# GROWTH

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# GROWTH

## A JOURNAL FOR STUDIES OF DEVELOPMENT AND INCREASE

VOLUME II, NUMBER 1

PAPERS 38-45

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# GROWTH

A journal for the coordination of studies of increase and development as general properties of nature. Published by contributors and subscribers on a cooperative, non-profit, non-salary basis. The field of investigation is primary and work will be published in which the approach is through mathematics, physics, chemistry, genetics, embryology, philosophy and anthropology, just as will studies of development in its several expressions, and of mass increase and the part played therein by nutrition, vitamines and hormones.

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# BODY PROPORTIONS IN THE GROWING INFANT

*By*

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(*Received Feb. 17, 1938*)

As part of the Yale Normative study of infancy, begun in 1927, data were secured concerning physical growth. Measurements were made of carefully selected infants for each of the 13 lunar month intervals for ages 8 through 56 weeks. This report is limited to a study of the growth changes in body proportions shown by the averages for successive lunar months. The individual growth curves will be analyzed after the cases have been remeasured at the age of 5 and 10 years.

The available data on physical growth in infancy have recently been reviewed by Bayley and Davis (1935). They comment on the paucity of norms for other than height, weight and circumferences. The studies which afford data comparable to any extent with those presented here are those of Schmid-Monnard[1] (1892), Bakwin and Bakwin (1931), Bayley and Davis (1935), Meredith (1935), and Boynton (1936). The last two authors give data for only four ages in infancy, 3, 6, 9 and 12 months. Reference will be made to the findings of the above authors when the techniques of measurement employed make the comparison justifiable.

The age norm values for the various measurements and indices have already been published (Gesell and Thompson, 1938) and the figures will not be reprinted here. The curves and data which are presented may be checked by reference to the prior publication where the full details of measurement procedures are also stated.

## THE SUBJECTS

The data include a total of 447 examinations of 101 different infants, 46 males and 53 females. The examinations were equally distributed over the age range so that there is a minimum of 24 records at any age level. Racially, the group is composed largely of Old Americans as designated by Hrdlička (1925), but it cannot quite claim that distinction since, although the parents were born in this country and were of northern European extraction, the grandparents were not all born here. All infants were cared for at home within a radius of a few miles from the center of New Haven. The homes were of average socio-economic status. The infants were all of full term, and were considered physically normal and healthy. *Examination was made within two days of the specified age.* The distinctions between age level groups were therefore well defined.

## THE MEASUREMENT PROCEDURES

The data include length from the soles of the feet to the vertex, to the suprasternal notch, and to the pubes;[2] transverse diameter of the thorax at the

[1] Data given by Bardeen, 1920.

[2] The term *pubes* is synonymous with *symphysion*. The authority for using *pubes* is Duckworth's report on the International Agreement for the Unification of Anthropometric Measurements to be made on the Living Subjects, 1912. See Hrdlička, Aleš 1920 *Anthropometry*, The Wistar Institute of Anatomy and Biology, p. 27.

nipples, and biacromial and bicristal diameters; circumferences of the head and of the thorax at nipple level; weight and dentition.

The longitudinal measurements were made by the use of a measuring board described in a previous publication (Thompson, 1929). Briefly the method of measurement was as follows: an assistant or the child's mother, following the examiner's direction, held the infant's lower extremities extended, with the soles of the feet pressed against the end of the board. The infant was moved or enticed into position in line with the anthropometer and with his Frankfort-Horizontal line vertical. The anthropometer arm was then moved down, measuring in turn the distance from soles to vertex, to suprasternal notch, and to pubes. Diameters and circumferences were measured in the usual standard manner; diameters with spreading calipers, and circumferences with a steel tape. All measurements were made with the infant reclining and supine.

## GROWTH TRENDS

The figures for the average, the minimum, the maximum, and the standard deviation for each age are given separately for the sexes in the publication previously cited (Gesell and Thompson, 1938).

The growth curves (Figs. 1, 2, and 3) picture the average trend of each measurement for both sexes. The male infants were found to be larger in each dimension measured than the female infants of the same age. The differences are usually representative of about one month's growth.

The slopes of the growth curves definitely decrease during the first half of the infancy period reaching a minimum between 24 and 40 weeks, following this period there is a slight increase in slope. The irregularity is slight but it is confirmed by a study of individual cases and also by an analysis of the monthly increments of the Bayley-Davis data on length for both male and female infants.

The temporary depression does not seem to be due to the selection of cases or to seasonal influences. It may be related to dentition since the slowing down of growth occurs at a time when dentition is rapid; for boys the slow growth occurs earlier than for girls and we note also that dentition occurs earlier in

---

FIG. 1. Average Measurements at 4-weekly intervals, ages 8–56 weeks. Longitudinal dimensions: a—soles to vertex; b—soles to suprasternal notch; c—soles to pubes. Solid line—boys; broken line—girls.

FIG. 2. Average Measurements at 4-weekly intervals, ages 8–56 weeks, Circumferences: a—head; b—thorax; c—diameters; d—biacromial; e—thorax; f—bicristal, Solid line—boys; broken line—girls.

FIG. 3. Average Measurements at 4-weekly intervals, ages 8–56 weeks. Weight and Dentition. Solid line—boys; broken line—girls.

FIG. 4. Growth trend of the ratio soles-pubes/total length. S–C: Scammon-Calkins' data, 1929. Solid line—boys; broken line—girls.

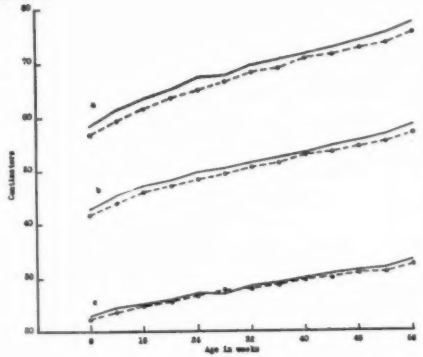
FIG. 5. Growth trend of the ratio, vertex-sternal/total length. S–C: Scammon-Calkins' data, 1929. Solid line—boys; broken line—girls.

FIG. 6. Growth trend of the ratio, sternal-pubes/total length. S–C: Scammon-Calkins' data, 1929. Solid line—boys; broken line—girls.

FIG. 7. Growth trend of the ratio, biacromial diameter/sternal-pubes length. S–C: Scammon-Calkins' data, 1929. Solid line—boys; broken line—girls.

FIG. 8. Growth trend of the ratio, thorax diameter/sternal-pubes length. S–C: Scammon-Calkins' data, 1929. Solid line—boys; broken line—girls.

FIG. 1



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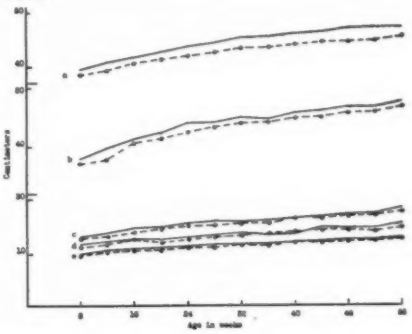

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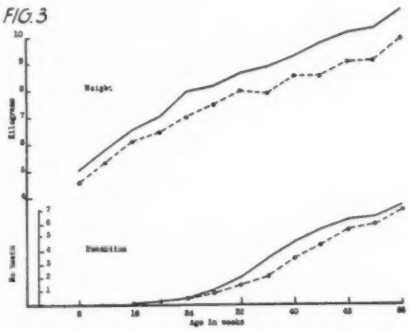


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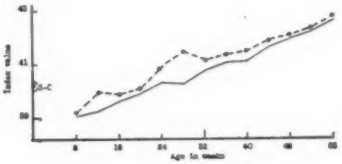


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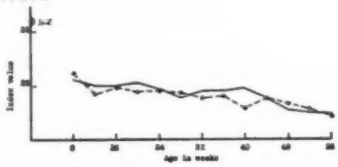
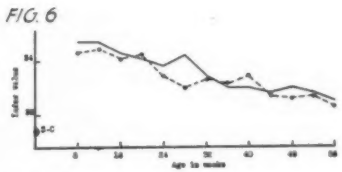



FIG. 6

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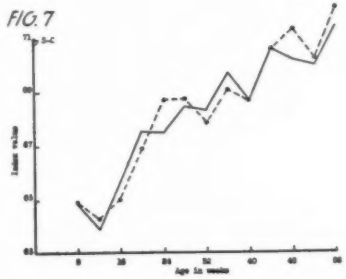


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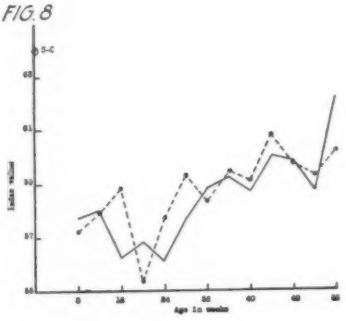


boys than in girls. A relation between dentition and growth is further suggested by the frequent association of poor appetite or digestive disturbance or a slight head cold with dentition. On the other hand the deviation may be merely a continuation of the decrease in growth process which is checked by some regulatory mechanism which stimulates growth as it reaches a low rate. In this case attention should be centered on the increase in slope as well as on the decrease.

## PROPORTIONATE GROWTH

The figures of table 1 give the increment and percentage increment gained by the different body dimensions during the period studied. We first note that

TABLE 1
INCREMENT AND PERCENTAGE INCREMENT OF GROWTH OF BODY PARTS BETWEEN AGES 8 AND 56 WEEKS

| Lengths | Increment | | Percentage increment | |
|---|---|---|---|---|
| | Boys | Girls | Boys | Girls |
| Soles to pubes | 10.5 cms. | 10.1 cms. | 46.3% | 45.5% |
| Soles to vertex | 19.7 | 19.0 | 33.0 | 33.5 |
| Pubes to suprasternal notch | 5.3 | 5.2 | 26.4 | 26.8 |
| Vertex to suprasternal notch | 3.9 | 3.7 | 25.5 | 24.3 |
| Diameters | | | | |
| Biacromial | 5.1 | 5.0 | 39.2 | 39.7 |
| Thorax | 4.2 | 3.7 | 36.2 | 33.3 |
| Bicristal | 3.2 | 3.0 | 33.0 | 31.6 |
| Girths | | | | |
| Thorax | 11.0 | 11.0 | 29.3 | 30.1 |
| Head | 8.3 | 7.7 | 21.3 | 20.2 |

the gains made by the boys are in every instance greater than the corresponding gains of the girls; but since the boys were larger to start with, the percentage increments for the boys and girls show no consistent difference. Whether or not the sex difference in the percentage increment for thorax and bicristal diameters is real or spurious cannot be determined from this data. However, the close correspondence of the percentage increments for boys and for girls in other dimensions and the consistent relative difference between thoracic and pelvic diameter

FIG. 9. Growth trend of the ratio, bicristal diameter/sternal-pubes length. S–C: Scammon-Calkins' data, 1929. Solid line—boys; broken line—girls.

FIG. 10. Growth trend of the ratio, bicristal diameter/thorax diameter. S–C: Scammon-Calkins' data, 1929. Solid line—boys; broken line—girls.

FIG. 11. Growth trend of the ratio, bicristal diameter/biacromial diameter. S–C: Scammon-Calkins' data, 1929. Solid line—boys; broken line—girls.

FIG. 12. Growth trend of a.—the ratio bicristal diameter/total length and b.—the ratio, bicristal diameter/soles-pubes length. S–C: Scammon-Calkins' data, 1929. Solid line—boys; broken line—girls.

FIG. 13. Growth trend of the ratio, biacromial diameter/thorax diameter. S–C: Scammon-Calkins' data, 1929. Solid line—boys; broken line—girls.

FIG. 14. Growth trend of the ratio, head circumference/total length. S–C: Scammon-Calkins' data, 1929. Solid line—boys; broken line—girls.

FIG. 15. Growth trend of the ratio, thorax circumference/total length ratio. S–C: Scammon-Calkins' data, 1929. Solid line—boys; broken line—girls.

FIG. 16. Growth trend of the ratio, head circumference/thorax circumference. S–C: Scammon-Calkins' data, 1929. Solid line—boys; broken line—girls.

FIG. 9

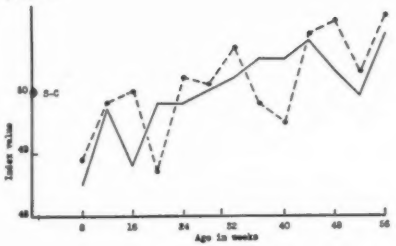
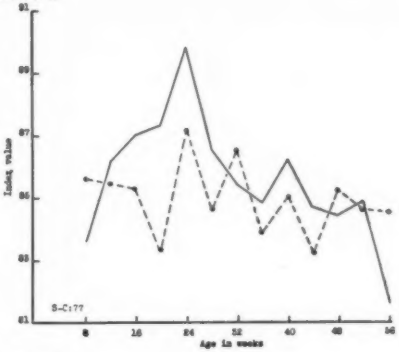



FIG. 10

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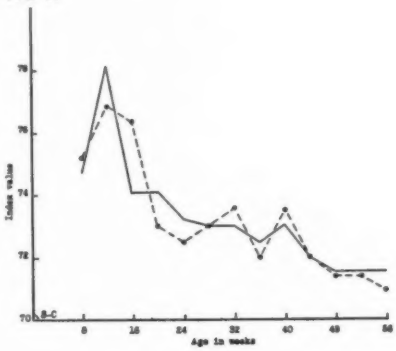


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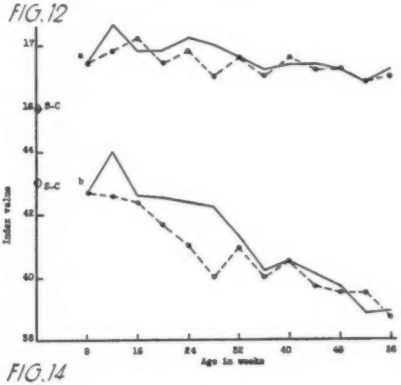
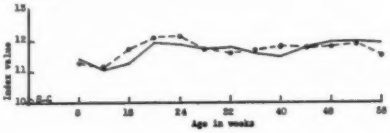



FIG. 13

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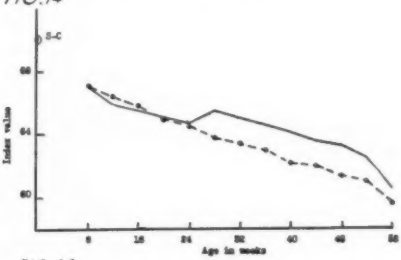
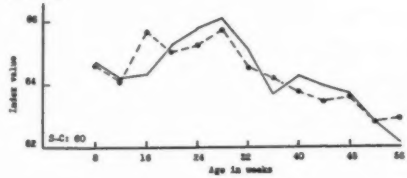



FIG. 15

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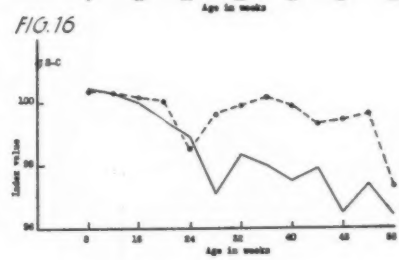

do permit us to draw several conclusions. In proportion to length at 8 weeks the soles-pubes length showed the greatest increase during the 48 weeks period studied. This contradicts the Bayley-Davis (1935, p. 45) statement, "It is only after the 12th month that the legs* begin to grow rapidly and cause a relatively greater increase in the total height curve." Just how Bayley and Davis reached their conclusion is not clear. Their data support the conclusion of this paper. The soles-pubes length and the shoulder breadth are both increasing more rapidly in proportion to length at 8 weeks than the body as a whole, i.e., the soles-vertex length.

The figures of table 1 also indicate that the soles-pubes length grew proportionately more rapidly than the sternal-pubes length, which in turn increased more rapidly than the vertex-sternal length. Also the thoracic girth proportionately increased more than the head girth demonstrating a similar trend of greater relative growth of the posterior parts. These findings, similar to those published by others, have been interpreted in terms of the law of developmental direction. The posterior parts are at an earlier stage of development and therefore are at a period of more rapid growth than the anterior parts.

However, when we inspect the figures for the transverse diameters we find the reverse growth trend. Shoulder breadth showed a greater percentage increase than thorax breadth which in turn showed a greater percentage increase than pelvic breadth. That is, the proportionate growth of the transverse diameters from shoulders to pelvis has been decreasingly rapid.

This apparent contradiction of the law of development direction may be a function of change in the relative position of bones rather than change in their dimensions. Our intensive individual growth studies show that the acromion rises from a position below the suprasternal notch at 8 weeks, at a position above it at 56 weeks. This rise may or may not serve to exaggerate the shoulder breadth growth. Similar changes might be operating with respect to transverse thorax growth.

There is another factor to be considered. Davenport (1935, p. 192) has pointed out that, "The widening of the shoulders depends in part on a change in the angle of the scapula with the frontal plane. From before birth to adolescence it tends to decrease from 60° to 30°." He also gives figures (p. 187) which indicate that at one month after birth this angle is about 30° which suggests that the change after the neonatal period is slight if not negligible. Theoretically, however, if the angle remains constant, a measured increase in biacromial diameter, unless accompanied by a reduction in anterior posterior dimensions, would be less than the actual increase in bone size.

It is obvious that deductions concerning the law of developmental direction, based on changes in diameter dimensions must be made with great caution. Surely the bicristal diameter gives an inadequate measure of the growth of the pelvic bone with its upward and outward flare.

* Legs in the Bayley-Davis study refer to total length minus vertex-buttocks length.

## AGE CHANGES IN BODILY PROPORTIONS

The manner in which the changes in bodily proportions occur is significant and not without possibility of misinterpretation. The trends of the various indices with age are pictured graphically.

*Longitudinal dimensions.* The first three comparisons relate segments of the longitudinal dimension to the infant's total length. The ratio for soles-pubes length (fig. 4) is increasing rather steadily; the ratio for vertex-sternal length (fig. 5) is decreasing slightly while that for sternal-pubes length (fig. 6) is decreasing more rapidly. It will be recalled that in proportion to size at 8 weeks, sternal-pubes length increased more rapidly than vertex-sternal length. It is a simple matter of arithmetic that a measure which increases in proportion to its size more than another will decrease more in proportion to total bodily size than that same measure, when and if the percentage increase of the total size is greater than the percentage increase of the parts.

The ratios for the Scammon-Calkins' (1929) data on birth measurements have been computed and added to the charts for reference. The vertex-sternal ratio to total length (fig. 5) shows a rapid decrease through 12 weeks, a slight decrease through 36 weeks, and thereafter a more rapid decrease during infancy.

The sternal-pubes length increases in proportion to total length prior to birth (Scammon and Calkins, 1929). It appears that the increase continues after birth (fig. 6) but that by 8 weeks at least a plateau is reached and by 12 weeks a decrease in the ratio begins and continues at least through infancy.

Likewise, the ratio of soles-pubes length to total length (fig. 4) appears to reach a critical minimum sometime after birth but prior to 8 weeks; thereafter the ratio increases rapidly. This proportion is of interest in that it shows a clear differentiation of the sexes. The soles-pubes dimension of the girl infant relative to length appears to be greater than the same measure for the boy infant. Whether the difference is a true expression of a sex difference in lower extremity growth is not clear from this study. Since we used the lower crease of the pubes as a landmark, differences in external genitalia may be operating to cause a consistent discrepancy. However, it would be expected that the discrepancy would favor a long soles-pubes length for the boy and it is not unlikely that the girl infant, who is on the average older than the boy infant of the same height, shows more mature body proportions.

*The transverse diameters.* It is natural to relate the transverse diameters to sternal-pubes length as well as to each other. Again ratios for birth measurements have been calculated and plotted on the figures.

Both the biacromial/sternal-pubes ratio (fig. 7) and the thorax diameter/sternal pubes ratio (fig. 8) decrease from their value at birth to a low point in the first half year and then increase. The shoulders reach the low point at an earlier age than the thorax; the shoulders at about 12 weeks, the thorax at about 20 weeks. The subsequent rise in index value is sooner for the shoulders than the thorax but after the rise occurs the curves are nearly parallel.

The course of the bicristal/sternal-pubes ratio (fig. 9) may be described as gradually increasing with fluctuations from birth on through 56 weeks. Com-

paring the pelvic diameter with thorax (fig. 10) and shoulder diameter (fig. 11) it is seen that the pelvis, in relation to both shoulders and thorax increases markedly from birth; the pelvis reaches a peak relative to the shoulders at 12 weeks and a peak relative to the thorax at 24 weeks. But we have just noted (fig. 9) that when compared with sternal-pubes length, the pelvis breadth changes only slightly. Also in proportion to total length, the soles-pubes length is practically constant (fig. 12,a). The increase in the proportionate size of pelvic breadth to shoulders and thorax is due principally to the fact that growth of shoulders and thorax decreases in relation to sternal-pubes length during the first half year of life and then increases, rather than to a relatively rapid growth of the pelvis as concluded by Knott and Meredith (1937, p. 324).

Also if we study the ratio of pelvic breadth to soles-pubes length (fig. 12,b) we find that the pelvis is broader in relation to legs at 12 weeks than at birth and that during infancy subsequent to 12 weeks the ratio declines. The decline must be attributed to acceleration in soles-pubes growth since the ratio of bicristal diameter/total length (fig. 12,a) changes very little after 12 weeks in comparison to the change in soles-pubes/total length ratio. The bicristal/soles-pubes ratio (fig. 12,b) tends to be greater for the boy infant than for the girl infant. Shall we assert then that the boy infant has a relatively larger pelvis than the girl infant? This statement while true is deceptive because the real difference lies in the fact that the girl infant has a relatively greater soles-pubes length than boys of the same total length (fig. 4) and it is this greater soles-pubes length which makes the pelvic breadth appear relatively smaller. It must be remembered that these growth ratios have two variables and that a change in a ratio may be due to either a relative increase in one variable or a relative decrease in the other variable.

The curve for the ratio, shoulder breadth/thorax breadth (fig. 13), shows a definite rise between 12 and 20 weeks and then, after a slight fall, is practically constant. This variation is attributable to the fact that the thorax is slower in broadening than the shoulders.

*The circumferences.* The head and thorax circumference will first be studied in relation to total length and then in relation to each other.

During the first year the head circumference of the girl infant is increasingly smaller in relation to total length (fig. 14). The head circumference/total-length index for the boy infant follows a similar course except that between 24 and 28 weeks there is an increase in relative head size. The discrepancy between male and female relative head size gradually decreases so that at 56 weeks it is slight. The data of Bayley and Davis (1935) and of Stuart (1934) reflect the same tendency though to a less degree. The Bayley and Davis data show the first definite sex differences at 4 months, while both the data of Stuart and those of the present study find a critical change at between 24 and 28 weeks. The operating factors are not obvious. The effect of hair growth on head circumference should produce as definite a variation in the ratio for the girl infant as for the boy infant but the curve for the girl infant apparently does not have the same critical point at any time in infancy.

The ratio, thorax-circumference/total-length (fig. 15), increases from birth to a maximum at 28 weeks and then decreases in value. The girl infant tends to reach a maximum at an earlier age than the boy infant. The data of this study are in closer agreement with those of Davenport (1923) than with those of Bayley and Davis (1935). If this index signifies chubbiness we would say with Davenport that at 28 weeks the body is at maximum chubbiness.

The height-weight indices have been published (Gesell and Thompson, 1938) but the curves are not included here since our data add no new facts. The significant question with respect to growth in weight concerns changes in weight distribution and we have no data on this point.

## RELATIVE GROWTH

Further analysis is necessary to determine whether the changes in body proportions which have just been reviewed are merely continuations of those insti-

TABLE 2
RELATIVE GROWTH RATES

| Relative to length: | | S–C | Age Interval—weeks | | | | | 56 weeks to 5 years |
|---|---|---|---|---|---|---|---|---|
| | | | 8–20 | 20–32 | 32–44 | 44–56 | B–B(4–52) | |
| Head circumference | B | .675 | .50 | .61 | .33 | .19 | .458 | .14 |
| | G | | .47 | .41 | .38 | .32 | | .12 |
| Thorax circumference | B | .6525 | .70 | .64 | .42 | .38 | .562 | .24 |
| | G | | .74 | .65 | .41 | .49 | | .21 |
| Biacromial diameter | B | .225 | .27 | .25 | .25 | .26 | .239 | .16 |
| | G | | .29 | .22 | .29 | .24 | | .18 |
| Thorax diameter | B | .2025 | .14 | .25 | .19 | .30 | | .06 |
| | G | | .14 | .27 | .29 | .12 | | .08 |
| Bicristal diameter | B | .1625 | .19 | .16 | .14 | .15 | .168 | .15 |
| | G | | .17 | .18 | .12 | .15 | | .15 |
| Vertex-sternal length | B | .3–.24 | .24 | .21 | .22 | .11 | | .13 |
| | G | | .17 | .22 | .24 | .17 | | .13 |
| Vertex-pubes length | B | .66 | .54 | .45 | .42 | .40 | | .34 |
| | G | | .52 | .45 | .44 | .44 | | .37 |
| Sternal-pubes length | B | .4–.28 | .30 | .25 | .19 | .30 | | .22 |
| | G | | .35 | .22 | .21 | .27 | | .24 |
| Soles-pubes length | B | .43 | .46 | .55 | .58 | .60 | (.545) | .66 |
| | G | | .49 | .55 | .56 | .56 | | .63 |

S-C Scammon-Calkins Constant for fetal growth.
B-B The Bakwin-Bakwin constant for infancy (4–52 weeks)
B Boys
G Girls

tuted at an earlier age or whether they are differential changes occurring during this period of growth.

Although they cite certain exceptions, Scammon and Calkins (1929, preface)

have concluded that, " . . . in general the linear growth of the various parts of the body is in uniform ratio during the fetal period . . . that changes in proportion arise, in spite of the constant fetal growth ratios, through inequalities already established in the earlier embryonic period."

Bakwin and Bakwin (1935) suggest that also during the first year of life a straight line relationship exists between various linear relationships and total body length and they have published constants appropriate for various dimensions.

The constancy of relative growth may be tested graphically, in which case measurements plotted according to length must conform to linear alignment, or arithmetically, in which case the ratio between the growth increment in any dimension and the growth increment for length during the same period must be constant. The time factor is eliminated in both analyses.

Using the data of the present study, relative growth rates have been computed for four consecutive 12-week intervals from 8 through 56 weeks for the various dimensions relative to total length. They are given in Table 2. The first column of the table lists the Scammon-Calkins' ratio for fetal growth; the next to the last column lists the constants for infancy determined by Bakwin and Bakwin; the intervening figures are ratios from our own data; and the last column gives similar ratios for the age period from 56 weeks to five years (Thompson, unpublished data).

TABLE 3
RELATIVE LOGARITHMIC GROWTH INCREMENTS

| Relative to total length: | | Age interval—weeks | | | |
|---|---|---|---|---|---|
| | | 8–20 | 20–32 | 32–44 | 44–56 |
| Head circumference | B | .75 | .93 | .54 | .30 |
| | G | .71 | .66 | .59 | .51 |
| Thorax circumference | B | 1.09 | .96 | .65 | .60 |
| | G | 1.12 | .85 | .66 | .79 |
| Soles-Pubes length | B | 1.17 | 1.36 | 1.43 | 1.42 |
| | G | 1.22 | 1.36 | 1.34 | 1.35 |

The ratios for both head and thorax circumference suggest a gradual decreasing relative growth rate for both dimensions. The fact that the trend is observable in both sexes reinforces the suggestion. The Bakwin-Bakwin constants appear to be an average of the ratios for this period.

For the biacromial and bicristal diameters there appears to be no defined trend. Perhaps more intensive study would show that the bicristal diameter has a larger relative growth rate index in early infancy as opposed to late infancy. The soles-pubes ratio is definitely increasing in value with age. For the other dimensions the course of the comparative growth rates is questionable and the difference may or may not be significant.

Huxley (1932, p. 249) points out that changes in body proportions may be

due not only to time handicaps of some parts over others but also to some parts "possessing a growth-coefficient above or below the body as a whole." In this case the growth curve is exponential in character according to the formula $y = bx^k$. Parts of the body following such a course of growth would have their logarithmic values, plotted against the logarithmic values for the body as a whole, lying on a straight line or would have the ratios between increases in logarithmic values for parts of the body and the body as a whole constant. Relative logarithmic growth increments have been computed for the three body dimensions whose relative growth rates were obviously not constant. (Table 3.)

The ratio for head circumference appears to be decreasing except for the value at the 20–32 week interval for the boys. It will be remembered that it was during this period that the growth curve for males showed an unexplained atypical trend. The ratio for thorax circumference likewise shows a decreasing trend. The soles-pubes length ratio to total length increases from the 8–20 weeks period and then remains constant for the boys after the 20–32 week interval and for the girls after the 8–20 weeks period.

These three dimensions, then, do not conform to Huxley's law of constant differential growth-ratio. The suggestion of Lumer (1927) concerning sigmoid growth of various types has not been investigated since the sigmoid function is particularly appropriate for growth over a long period of time and infancy is a relatively short period.

Nevertheless the growth data have been sufficiently analyzed to show that the body changes in infancy are not explainable in terms of a few laws of growth. Such an explanation is not only misleading but erroneous. When we consider all of the possible growth modifying factors of the environment, the interaction of growth accelerating hormones of the body, and the dynamics of growth of the body tissues themselves, is it any wonder that the body growth of the infant does not reduce to simple formulae?

## SUMMARY

Growth data on the body measurements of a normal, healthy, homogeneous group of white infants for the ages 8 through 56 weeks of age show a slowing down of growth of all dimensions during mid-infancy which may be related to complications of dentition. At each month females are about 4 weeks behind the males with respect to every dimension. However, the percentage increments of growth for female dimensions equals that for male dimensions except with respect to thorax and pelvis; for both of these dimensions the males have a greater percentage increase for the period studied. Longitudinal dimensions and circumferences change according to the law of developmental direction but the growth of the transverse measurements of shoulders, thorax and pelvis show a reversal of the trend. Change in relative position of the ribs and bones of the shoulder girdle may or may not explain the discrepancy.

The body proportions of the infant during the 8–56 weeks age period change in various ways. Relative to total length, the soles-pubes length increases rapidly, the sternal-pubes length decreases and the vertex-sternal length decreases

slightly. In proportion to sternal-pubes length, the shoulder breadth decreases to a low point at 12 weeks and then increases rapidly. The thorax decreases to a low point at 20 weeks and then increases similarly to the shoulders, while the pelvic breadth increases only slightly with fluctuations. In proportion to the pelvis, the shoulders broaden after the age of 12 weeks and the thorax broadens after 24 weeks.

The body proportion which shows the most regular change with age is the soles-pubes/total length ratio. This ratio also differentiates the sexes. The ratios, bicristal diameter/total length and biacromial diameter/thorax diameter change the least with age. They may therefore be helpful in distinguishing body types. In general, all other body proportions show such definite changes with age in infancy that any profitable study of consistency of body types in growth must relate each individual to his age norm.

Relative growth ratios for the 4 consecutive intervals of 12 weeks show that changes in body proportions are not merely the result of inequalities established at an early age. Furthermore the relative logarithmic growth ratios indicate that the changes are not merely due to one part having a growth coefficient different from another part. We must conclude instead that physical growth in infancy is undergoing modifications from an indefinite number of sources.

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# THE RELATION BETWEEN WATER AND DRY SUBSTANCE IN THE BODY OF THE RAT, BEFORE AND AFTER BIRTH

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The following relationships seem to exist between the body water and growth: at the time when growth is rapid, the normal body contains more water than later when growth is slow; the body of the fetus contains a higher percentage of water than that of the child, and the latter is richer in water than the body of the adult. Secondly, the rate of growth may be slowed down, stopped or reversed by an insufficient water intake, that is, by thirst. The action of thirst is partially indirect, in that it leads to a refusal of food, as described by all investigators attempting to produce pure dehydration (see, for instance, Kudo '20); if, however, a dry food is taken or forced, the body proteins are broken down at a more than normal rate, as indicated by a rise in nitrogen excretion (Straub '99, Dennig '98, Landauer '95). Finally, it seems possible that when growth is slowed down due to infections or other causes the new tissues formed may be poor in interstitial water (Hamilton and Shock, '34), and the loss of tissue water is one of the striking changes taking place in starvation (Gamble et al, '23). It is possible that the inadequate growth observed on a low caloric intake, on a qualitatively deficient diet, in chronic infections or in endocrine disturbances is of the same composition as normal growth, but it is also possible that it may differ from the normal and that the various forms of inadequate growth may differ from each other in respect to composition.

The reason why the water content of the body was chosen as the subject of this study is that in clinical work with infants one so often gains the impression that one factor of great importance in poor growth is the inability of the body to retain sufficient water. The chronically ill infant sometimes becomes dehydrated on rather slight provocation, and it seems as if in these cases the reserves of body water were either not at their normal level or depleted more easily than is normally the case. One may also see cases of poorly growing infants where repeated parenteral injections of fluids lead to a permanent gain in weight. Observations of the kind now mentioned emphasize the relation that undoubtedly exists between growth and body water, between the increase in protoplasm and its fluid environment.

In order to study abnormal variations in the composition of growth, it is necessary first to obtain, as exactly as possible, figures showing the range of normal variations. The water content of the rat fetus has been determined by Gonzales ('32) but the degree of variation is not given. With the exception of the youngest feti, the mean values for water content agree well with ours. In

the postnatal period, the water content of rats has been studied by Lowrey ('13) whose data for dry substance of the whole body (with the exception of those obtained in the new-born) were not obtained by drying the whole animal, but by a summation of the dry substance of various parts. The maxima and minima for each age group are given, but no statistical estimate of the degree of variability. The data of y Gonzales as well as those of Lowrey are shown in Table I together with our own.

TABLE I. PERCENT OF WATER IN WHOLE BODY

| Age, days | y Gonzales* | Lowrey | Authors** | Coeff. of Variation† | Calculated from Equation 16 |
|---|---|---|---|---|---|
| 13 (fetal) | 95.0 | | | | |
| 14 | 93.1 | | 92.3 | 0.23 | 92.8 |
| 15 | 93.0 | | 92.1 | 0.22 | 91.9 |
| 16 | 91.4 | | 91.5 | 0.71 | 91.1 |
| 17 | 90.7 | | 90.8 | 0.26 | 90.3 |
| 18 | 90.4 | | 90.0 | 0.51 | 89.6 |
| 19 | 89.5 | | 89.3 | 0.91 | 88.9 |
| 20 | 87.9 | | 88.2 | 0.30 | 88.3 |
| 21 | 87.4 | | | | |
| Birth | 86.9 | 88.3 | 87.1 | 1.2 | 87.4 |
| 2 | | | 83.2 | 1.4 | |
| 4 | | | 82.2 | 0.9 | |
| 6 | | | 81.4 | 2.5 | |
| 7 | | 79.9 | | | |
| 8 | | | 79.5 | 3.1 | |
| 10 | | | 76.7 | 3.0 | |
| 20 | | 70.1 | | | |
| 30 | | | 73.5 | 0.6 | |
| 42 | | 70.5 | | | |
| 60 | | | 70.5 | | |
| 70 | | 67.0 | | | |
| 90 | | | 70.0 | | |
| 150 | | 67.8 | | | |
| Adult | | 68.5 | 66.8 | | |

* The author's figures have been recalculated to correspond to even 24-hour intervals.
** The number of animals in each group is given in Table 2.

† $100. \frac{\text{Standard deviation}}{\text{Mean}}$.

The table shows a rather remarkable constancy in the water content of the fetus, in each age group. It should be pointed out that such lack of variability may, perhaps, only be expected when the measurements are made by one and the same technique, by one person, on one stock of animals. After birth the variations between different animals become more marked.

By suitable interpolation, one may from Table I obtain the approximate percentage of water at any age and also, at least for the younger age groups, the degree of variation of these percentages. However, we are not primarily interested in the relation between water content and age, but in the relation between the rate of increase of dry substance to the rate of increase of water. Needham ('34) using Lowrey's ('13) and y Gonzales' ('32) figures has shown that the relation between water and dry substance in the rat as in many other animals,

may be expressed by an equation $y=bx^k$, where $b$ and $k$ are constants. This equation may, of course, also be written: $\log y = k \log x + \log b$ and any variables showing this relationship will fall on a straight line when plotted logarithmically. The equation indicates that there is a constant ratio between the relative growth rates of the two factors; the "constant differential growth ratio" of Huxley ('32). In mathematical terms:

$$\frac{dy/y}{dx/x} = k.$$

FIG. 1. The relation between the logarithm of water and the logarithm of dry substance, from 14 days of fetal age to adult life.

In figure 1 the logarithms of the values for water have been plotted against the logarithms of the values for dry substance. This figure includes all our data, while in Table II are shown the mean values for each time period. The variability of the values is, no doubt, chiefly due to the fact that the determination of fetal age is rather uncertain. The degree of variability of these figures is, therefore, of less interest than the degree of accuracy of the means; that is the reason why the standard error, not standard deviation, is given in Table 2. The values in the table are for the sake of brevity, given in grams, while in all other instances the values are expressed in milligrams. In calculating equations the mean values have always been used, as they are more exactly related to age than the individual data; calculating from individual data gives, however, practically the same relationships.

It may be seen in figure 1 that there is approximately linear relationship between weight of water and weight of dry substance when plotted logarithmically. There is, however, a break in the line at birth, and after birth the

irregularities in the line are more marked than during the fetal period. On the whole, however, the linear relationship must be considered quite good.

TABLE II

| Age Days | Number of Animals | Number of Groups | Dry Subst. Grams | Standard Error | Water Grams | Standard Error | Total Body Weight, Gms. | Standard Error |
|---|---|---|---|---|---|---|---|---|
| | | | | Foeti | | | | |
| 14 | 109 | 11 | 0.0055 | 0.0003 | 0.066 | 0.004 | 0.072 | 0.004 |
| 15 | 53 | 5 | 0.0119 | 0.0004 | 0.139 | 0.005 | 0.151 | 0.005 |
| 16 | 94 | 11 | 0.0228 | 0.0008 | 0.246 | 0.009 | 0.268 | 0.010 |
| 17 | 46 | 5 | 0.0466 | 0.0004 | 0.459 | 0.009 | 0.506 | 0.009 |
| 18 | 92 | 10 | 0.094 | 0.008 | 0.84 | 0.06 | 0.93 | 0.07 |
| 19 | 43 | 5 | 0.172 | 0.027 | 1.42 | 0.17 | 1.59 | 0.19 |
| 20 | 99 | 10 | 0.273 | 0.008 | 2.04 | 0.05 | 2.31 | 0.06 |
| Birth (21.5) | 49 | 13 | 0.73 | 0.02 | 4.9 | 0.1 | 5.7 | 0.1 |
| | | | | After Birth | | | | |
| 2 | 43 | 12 | 1.10 | 0.05 | 5.5 | 0.3 | 6.6 | 0.3 |
| 4 | 38 | 11 | 1.46 | 0.05 | 6.8 | 0.3 | 8.2 | 0.3 |
| 6 | 36 | 15 | 2.2 | 0.1 | 9.6 | 0.3 | 11.8 | 0.4 |
| 8 | 30 | 13 | 3.2 | 0.2 | 12.3 | 0.4 | 15.5 | 0.6 |
| 10 | 16 | 11 | 4.7 | 0.4 | 15.5 | 0.7 | 20.0 | 1.0 |
| 30 | 8 | 8 | 17.1 | 0.5 | 47.0 | 1.0 | 65.0 | 2.0 |
| 60 (males) | 3 | 3 | 59.0 | | 146.0 | | 205.0 | |
| (females) | 3 | 3 | 45.0 | | 103.0 | | 148.0 | |
| 90 (males) | 3 | 3 | 76.0 | | 185.0 | | 261.0 | |
| (females) | 3 | 3 | 57.0 | | 128.0 | | 185.0 | |
| Adult, male | 1 | 1 | 146.0 | | 289.0 | | 435.0 | |
| female | 1 | 1 | 91.0 | | 186.0 | | 276.0 | |

The relationship between water and dry substance is, then, of the type: $\log y = k \log x + \log b$, where $y$ is water, $x$ dry substance, $k$ and $b$ constants. The fetal data, including the values at birth, conform to the equation:

(1) $$\log_e y = 0.875 \log_e x + 2.745, \quad \text{or}$$

(2) $$y = 15.6x^{0.875}.$$

The standard error of equation 1 is $\pm 0.025$; the correlation coefficient between $\log x$ and $\log y$ is $+0.9999 \pm 0.00007$; the standard error of the regression coefficient 0.875 is $\pm 0.004$. This coefficient is, of course, the constant $k$ of Huxley ('32) (the coefficient of growth partition) indicating the degree of heterogony between the increase of water and dry substance. Differentiating equation 1 we obtain:

(3) $$\frac{dy/y}{dx/x} = 0.875,$$

showing that there is a constant ratio of 0.875 between the relative growth rates of water and dry substance.

The relation between the values for water ($y$) and dry substance ($x$) found after birth may be expressed by the equations:

(4) $$\log_e y = 0.82 \log_e x + 2.81;$$

(5) $$y = 16.6x^{0.82}.$$

(6) $$\frac{dy/y}{dx/x} = 0.82.$$

The standard error of equation 4 is $\pm 0.058$; the correlation coefficient between $\log y$ and $\log x$ is $0.999 \pm 0.0005$; the standard error of the regression coefficient 0.82 (the coefficient of growth partition) is 0.01. This coefficient is significantly different from the coefficient of 0.875, obtained for the fetal values, as the difference between the two coefficients is about five times as great as the error of this difference.

Equations 3 and 6 indicate that there should be a constant ratio between the relative growth rates of water and of dry substance. How far that is true in the time intervals at which our data were obtained, is shown in Table III. In this

TABLE III

| Fetal Age Days | Percentage Growth Rates $\frac{100\Delta \log_e x}{\Delta t}$ | | | Ratio Between Percentage Growth Rates of Water and Dry Substance $\frac{\Delta \log_e y}{\Delta \log_e x}$ |
|---|---|---|---|---|
| | Dry Substance | Water | Total Body | |
| **Foeti** | | | | |
| 14–15 | 77.1 | 74.1 | 74.4 | 0.96 |
| 15–16 | 65.1 | 57.1 | 57.7 | 0.88 |
| 16–17 | 71.4 | 62.6 | 63.4 | 0.88 |
| 17–18 | 69.7 | 60.5 | 61.4 | 0.87 |
| 18–19 | 61.2 | 52.4 | 53.1 | 0.86 |
| 19–20 | 46.0 | 36.2 | 37.3 | 0.79 |
| 20–21.5 (birth) | 65.5 | 59.0 | 60.0 | 0.90 |
| **After Birth** | | | | |
| Birth–2 | 25.6 | 5.1 | 7.4 | 0.20 |
| 2–4 | 14.0 | 10.7 | 11.6 | 0.77 |
| 4–6 | 20.9 | 17.4 | 18.1 | 0.83 |
| 6–8 | 18.5 | 12.5 | 13.6 | 0.68 |
| 8–10 | 19.6 | 11.4 | 13.3 | 0.58 |
| 10–30 | 6.4 | 5.6 | 5.8 | 0.88 |
| 30–60 (males) | 4.1 | 3.7 | 3.8 | 0.90 |
| (females) | 3.2 | 2.6 | 2.8 | 0.81 |
| 60–90 (males) | 0.84 | 0.80 | 0.81 | 0.95 |
| (females) | 0.85 | 0.71 | 0.76 | 0.84 |
| 90–adult (males) | | | | 0.67 |
| (females) | | | | 0.82 |

table the ratios between relative growth rates have been estimated by calculating $\Delta \log_e y / \Delta \log_e x$ for each successive pair of means. During the fetal period there is fairly good agreement with the ratio given in the equations, while after birth there are some marked variations, especially in the period immediately following birth, and also in the older males. Probably the post-natal growth in respect to water and dry substance should be divided into several periods.

The relation between water and dry substance in the fetus may be derived

from two other relationships: between water ($y$) and fetal age ($t$ days*) and between dry substance ($x$) and fetal age.

(7) $$\log_e y = 9.81 \log_e t - 21.67;$$

(8) $$\log_e x = 11.20 \log_e t - 27.88, \quad \text{or,}$$

(9) $$y = 10^{-10} \cdot 3.898\, t^{9.81};$$

(10) $$x = 10^{-13} \cdot 7.844\, t^{11.21}.$$

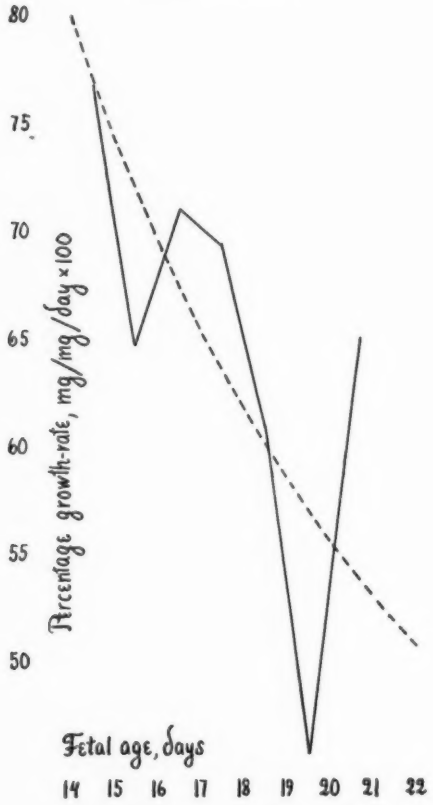


FIG. 2. The relative growth rate of dry substance (solid line) as compared to calculated values (broken line).

Equation 7 has a standard error of $\pm 0.062$, equation 8 of $\pm 0.054$. The correlation coefficient between $\log_e y$ and $\log_e t$ is $0.999 \pm 0.001$; between $\log_e x$ and $\log_e y$ $0.999 \pm 0.0007$.

* As the pregnancy of the rat lasts for 21–22 days, we have assumed that the age of birth is 21.5 days (Long and Evans ('22) give the average age as 21.8 days).

Eliminating $t$ from equations 7 and 8, we obtain equation 1.

Differentiating equation 7, we obtain:

$$\frac{dy}{y} = 9.81\frac{dt}{t};$$

$$\frac{dy}{y}\cdot\frac{1}{dt} = 9.81\frac{1}{t}. \tag{11}$$

Similarly, we obtain from equation 9:

$$\frac{dx}{x}\cdot\frac{1}{dt} = 11.20\frac{1}{t}. \tag{12}$$

Calculating the relative growth rates from the data (by calculating $\Delta \log_e y/\Delta t$ and $\Delta \log_e x/\Delta t$ for each successive pairs of means) we find (figure 2) that equations 11 and 12 express just a general trend, and that the curve indicating changes in the actual relative growth rates has a tendency to oscillate back and forth across the calculated curve.

The total body weight, $z$, is the sum of water and dry substance, $y+x$. Examining the relation of total body weight to age, we find:

$$\log_e z = 9.93 \log_e t - 21.91; \tag{13}$$

$$z = 10^{-10}\cdot 3.067 t^{9.93}; \tag{14}$$

$$\frac{dz}{z}\cdot\frac{1}{dt} = 9.93\frac{1}{t}. \tag{15}$$

Equation 13 has a standard error of 0.062. The correlation coefficient between $\log_e z$ and $\log_e t$ is $0.999 \pm 0.0007$.

The three equations describing the relation of water ($y$), dry substance ($x$) and total body weight ($z=y+x$) to fetal age ($t$) cannot, of course all three of them be true for all values of $t$. When in this instance all three equations are approximately true this is due, first to the fact that $y$ makes a much larger contribution to $z$ than does $x$; so that the relation between $z$ and $t$ largely reflects that between $y$ and $t$. Another factor is the rather slight degree of heterogony between $x$ and $y$, in this instance expressed by the constant 0.875. If this constant had been equal to 1, then all three equations could be true for all values of $t$. For, if

$$\log y = \log x + \log a;$$

$$\log y = c_1 \log t + \log b_1$$

$$\log x = c_2 \log t + \log b_2$$

where $a$, $b_1$, $b_2$, $c_1$, $c_2$ are constants, then, eliminating $\log t$ from the last two equations, one gets:

$$\log y = \frac{c_1}{c_2}\log x + K, \text{ where } K \text{ is a constant.}$$

Then,

$$\log x + \log a = \frac{c_1}{c_2} \log x + K$$

$$(c_1/c_2 - 1) \log x = K_1, \text{ where } K_1 \text{ is a constant.}$$

As $x$ is a variable, $c_1$ must equal $c_2$. The two equations relating $y$ and $x$ to $t$ may then be written:

$$y = b_1 t^{c_1}$$

$$x = b_2 t^{c_1}, \text{ and adding these two equations,}$$

$$y + x = z = b_3 t^{c_1}, \quad \text{where} \quad b_3 = b_1 + b_2.$$

If, each of the two parts, $x$ and $y$, has a straight line logarithmic relationship to $t$, then one may expect the sum of the two parts ($z$) also to have approximately such a relationship when (1) the two parts are of very unequal magnitude, so that one of them is nearly equal to the total, $z$, and (2) when the heterogony between the two parts is not very marked so that the heterogony constant is close to unity. In our case, both these conditions are present.

The relationships between water and time and total body weight and time may be used to obtain a formula expressing the relationship between percentage of water and fetal age. If $y$ is water, $z$ total body weight, then the percentage of water, $P$, is $100\ y/z$. From equations 9 and 14, we may then get an equation of the type $P = ct^p$, from which $P$ may be calculated with an accuracy of 0.8 or less. However, an even better formula with slightly different constants may be obtained by directly calculating the best linear relationship between $\log P$ and $\log t$:

(16) $$\log P = -0.1388 \log t + 2.1265$$

(17) $$P = 133.8\, t^{-0.1388}.$$

By the means of these equations the mean percentage of water for a certain fetal age (from 14 days up to and including birth) may be calculated with an accuracy of 0.5 or less (Table 1).

It is evident from several of the equations given above, that they cannot be valid at $t = 1$. Equation 17 illustrates that fact perhaps better than any of the others, because at $t = 1$ we obtain a water content of 133.8 percent.

After birth, the values for water and dry substance, if plotted logarithmically against $t$, show a roughly linear relationship, which, however, is not as good as that obtained from the fetal values. As the period after birth is being subjected to further study, we shall, however, not discuss it at the present time.

*Discussion.* It appears from the above, that the changing water content of growth during the fetal period studied may be suitably expressed as a ratio ($k$) between the relative growth rate of water and the relative growth rate of dry substance; and that this ratio remains fairly constant, if one calculates the growth rates for one-day periods. After birth, it is possible that the change in

water content is better expressed some other way, but according to our data, this simple way of expressing the facts will, probably, be found useful also in the study of post-natal growth. It is obvious, however (see Table 3), that if the drying-out process is expressed as a ratio between the relative growth rates of water and dry substance, then post-natal growth should be divided into several periods. It also seems probable that the same thing would have to be done for fetal growth if it could be studied from the first division of the egg.

With the material now available, the following growth periods in respect to water and dry substance, are apparent:

1. The last week of fetal life (14 days up to and including birth). During this period, taken as a whole, $k$ is 0.875.

2. The first ten days after birth. There are great variations of $k$ during the two-day periods covered by the data during this time. The $k$ for the whole period is 0.61, that is, the body is getting comparatively more dry at a very rapid rate. The greatest decrease in water content is during the first two days (see table 1 for percentages of water and table 3 for $k$), but even if this first two-day period is excluded, the rest of the period has a $k$ of only 0.71.

3. From ten days on the data are not numerous enough and do not come sufficiently close to each other in time to allow any definite conclusions. However, it is possible that no further division into periods is needed. The next values after ten days were obtained at thirty days, at which age the water content of eight animals was determined. Calculated for the period 10–30 days, $k$ was found to be 0.88, that is, nearly exactly the same value that was found during the fetal period. From thirty days on we have only three animals or less in each group, and the mean values may, therefore, be far from exact. However, the values for $k$ during the periods following the age of thirty days are, with one exception, between 0.81 and 0.95, which makes it seem possible that the actual mean rate of drying-out at this age may be the same as in the period 10–30 days. If that is so, then $k$ would be approximately constant from 14 days of fetal age to the cessation of growth, with the exception of a short period after birth.

We have in the preceding paragraphs discussed water and dry substance and demonstrated certain relationships that exist between them. Both water and dry substance are complex factors from an anatomical standpoint. There are several kinds of body fluid (cellular, interstitial, lymph, serum) and several kinds of dry substance. The steady decrease in water content, then, is a complex phenomenon, and it may take place in three ways:

1. Tissues containing much water may grow more slowly than tissues containing smaller amounts of water. Fatty tissue is poor in water content, and during the first week of life there is a marked increase in the percentage of body fat (Hatai '17). Another factor affecting the percentage of body water in the same direction may be the calcification of bones. The marked increase in dry substance during the first ten days of life may be due to these phenomena, but it seems unlikely that any difference in growth rates of various tissues can explain the steady decrease in water content of the whole body during the whole period of growth.

2. There might be a proportional decrease in the water content of all tissues and tissue components.

3. There might be a decrease in the amounts of interstitial tissue water, while the cellular water remains in a more constant relationship to the amounts of cellular tissue.

*Conclusions.* The ratio between the relative growth rates of water and dry substance in the body of the rat, is approximately constant from 14 days of fetal life to birth. The values for water and dry substance conform to an equation: $y = bx^k$, where $y$ is water, $x$ dry substance and $b$ and $k$ constants. This equation may be derived from two others, between water and fetal age ($t$) and between dry substance and fetal age, both of the form $y = ct^p$, where $c$ and $p$ are constants. The total body weight, which is the sum of water and dry substance, also has the same kind of relationship to $t$. It is obvious, that all three of these relationships cannot be true for all values of $t$, but (for reasons explained in the text) it happens that they are all very nearly true during the age period in question.

During this fetal period, the mean percentage of body water ($P$) for a certain age ($t$) may be very accurately determined from the equation: $P = 133.8t^{-0.1388}$.

From birth to the cessation of growth the values for water and dry substance also conform, approximately, to an equation of the form $y = bx^k$, but this equation is not as accurate as the one obtained for the data on the fetus. Probably post-natal growth should be divided into several periods. With the data now available it seems that during the first ten days after birth the relative growth rates are very irregular from day to day, and that on the whole, during this period the drying-out of the body proceeds at a more rapid rate than during the preceding fetal period. Then follows a period, from ten to thirty days, during which, on the average, growth in respect to water content proceeds exactly as during the last week of fetal life. It seems possible that this type of growth continues to adult life.

## METHODS

*Method of Determining Fetal Age.*—The oestrous cycle in each rat was followed by means of vaginal smears using the criteria of Long and Evans ('22) to determine the beginning of heat. When the females were observed to be in heat they were placed in the cage with the males late in the day and allowed to remain there overnight. The following morning vaginal smears were again made on each female and only those in which spermatozoa were actually found in the vagina were considered mated. These were immediately separated from the males. The day on which the spermatozoa were observed was counted as the first day of gestation. Evans and Long (page 33) have pointed out that at least four hours elapse between copulation and fertilization and as much as thirty-three hours may elapse.

*Preparation of Fetal Material.*—Before the 14th day of gestation the embryos were so small and so fragile that it was impossible to separate them from the placenta with any accuracy. Therefore, the youngest age group was taken at that time.

The pregnant rats were killed with ether, the uterus opened and the amniotic sack ruptured. The umbilical cord was clamped and the fetus removed and quickly dried by blotting with small pieces of filter paper. The feti taken from each rat were weighed as a group. They were dried at 100°C to constant weight.

*Preparation of Rats.*—No attempt was made to relate the time at which the rats were killed to the time of feeding. Food was before the animals at all times. They were killed by ether, the skin cut in several places and the body cavities opened to facilitate drying and dried to constant weight at 100°C.

We are greatly indebted to Dr. Sewall Wright for advice and criticism.

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# THE EFFECT OF SUPRAMAXIMUM TEMPERATURES ON THE DEVELOPMENT OF RANA PIPIENS

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## SUMMARY

*When eggs of Rana pipiens or Rana sylvatica are raised at slightly supramaximal temperatures, closure of the blastopore is delayed with the result that a tail bud is formed at the dorsal margin of the open blastopore. The subsequent development of such larvae of Rana pipiens includes an atypical closure of the blastopore which is accompanied by an extrusion of yolky material, and usually results in some degree of microcephaly. The microcephaly is associated with an atypical development of the anterior foregut region, a developmental arrest of the dorsal medullary structures often including the absence of the eyes, and the suppression of various ectodermal organs of the facial field. In the trunk region the chief modifications are associated with the development of the neural tube, the notochord, the gut, and the myotomes. The precursors of the myotomes often form fused units which differentiate as transverse muscle masses. At the higher temperatures the utilization of the yolk is not carried on as in the control. A small percentage of the larvae which have previously shown the delayed closure of the blastopore subsequently recover and become essentially normal larvae. A comparison of the effects of supramaximal and subminimal temperatures is made and the conclusion is drawn that temperature affects the processes concerned with morphogenesis and those concerned with embryonic segregation differentially.*

The following report contains a description of the effects of temperatures which are slightly supra-maximal (for the whole development) upon the early development of the frog, Rana pipiens. Lillie and Knowlton (1897) have defined the limits of development as follows: "the minimum temperature for the whole of development is the lowest and the maximum the highest at which normal development may occur." Between the maximum and the minimum, changes in temperature do not affect the rates of the developmental processes sufficiently to preclude the formation of normal individuals. Outside of these limits, alterations in the gross structure are encountered which in some cases may be shown to be directly dependent upon initial differences in rate not compensated for during the later developmental stages. Lillie and Knowlton have called attention to the fact, that, while cell division may proceed in an apparently normal fashion in all parts of the germ, there are differences in the relations between the rates in the animal and vegetative hemispheres of the amphibian egg at both extremes of the temperature range. Attention should be called to the fact that maximum and minimum temperatures for isolated periods

in development and consequently for individual developmental processes may differ from those which are limiting for the whole of development (cf. Hoadley and Brill, 1937).

The use of modification of temperature in the analysis of development is based on the hope that the processes concerned may be differentially affected and that for that reason it may be possible to describe them and to define the role they play (see Needham, 1933; Tyler, 1936a and b; Hoadley and Brill, loc. cit). Over by far the greater part of the temperature range the development of the germ conforms to the normal pattern insofar as the whole of development is concerned and the implication is that this signifies an equal or proportional effect upon the processes involved. Such a conclusion is not justified for it has been found by numerous workers that within relatively wide limits both quantitative and qualitative defects may be compensated for by regulation at later stages of development. Above the maximum and below the minimum the regulation is not complete and consequently individuals are formed which display certain specific defects. Not far above the maximum and below the minimum, temperatures are reached which completely suppress cell division and hence the gross morphological evidence of development.

The effect of sub-minimum temperatures on the development of the frog's egg has been discussed by O. Hertwig(1894, '95), and by Schultze ('95), and that of supra-maximal temperatures by O. Hertwig. Sub-minimal temperatures gave rise consistently to larvae showing spina bifida. Supra-maximal temperatures, on the other hand gave rise to larvae which showed, not a spina bifida, but an axial development from the margin of the blastopore which, in turn, remained open. Hertwig compares the formation of these larvae to that of embryos of the elasmobranch. The analysis is far from complete and concerns only the earlier stages. The general picture presented by larvae raised under the conditions of the two temperature extremes is very different and deserves further consideration. The larvae raised at supra-maximal temperatures are particularly interesting inasmuch as they resemble those obtained by other workers using a variety of methods, and when compared with those obtained at the low temperatures show a differential effect on the processes of cytoplasmic differentiation (i.e. histogenesis) as compared with those of morphogenesis. The following methods have yielded results which may be compared with those obtained by the use of extremes of temperature and hence should be mentioned; NaCl solutions (Morgan and Tsuda 1894; Hertwig 1895), LiCl solutions (Lehmann 1937a and b; Cohen 1938), dilute Ringer's solution after removal of the membranes of the blastula (Holtfreter 1933a and b), cane sugar solutions (Piiper 1933), thyroxin solutions (Bauman 1936), 2,4-dinitrophenol (Dawson 1938), aging of the eggs before insemination (Witschi 1930), and X-ray (Curtis, Cameron, and Mills 1936).[1] The results may be roughly classified as follows:

[1] Open blastopores resembling some of those mentioned above have been described by Wintrebert (1934). They were obtained by the asphixiation of the vegetative hemisphere of the developing egg of Discoglossus pictus.

complete exogastrulation, persistent yolk plug with spina bifida, and persistent yolk plug with marginal development of the axial portion of the embryo. In the second and the third types the yolk plug may eventually close with the extrusion of yolky substances. The third type of behavior is that characteristic under conditions of supra-maximal temperatures.

Preliminary results were obtained in the present investigations with the eggs of Rana sylvatica; subsequent tests were made with the eggs of Rana pipiens obtained according to the method described by Rugh (1934). The general behavior of both forms was the same. After 27 to 32 hours of development the chief modifications in the developmental picture are to be found in the relation between the tail bud and the open blastopore, in the development of the mesoblast, especially in the blastoporic region, and in the development of the notochord and the endodermal tube. After $64\frac{1}{2}$ hours considerable regulation has taken place resulting in closure of the blastopore usually following extrusion of some of the yolky material and in the formation of a typical hindgut and anus. The anus in one case may have developed directly from the closing blastopore. Defective or atypical development is to be found in the notochord, the heart, the gut, and in the arrangement of the muscle bands of the somites. There is ample evidence that the yolk is not utilized as in the control. There is a reduction in the extent of the foregut with which may be associated the absence or extensive suppression of the eyes, the tendency toward fusion of the suckers and nasal regions, and the microcephaly which in many cases is extreme. The membranous labyrinth of the internal ear remains cystic though imbedded in mesenchyme. The development of the anterior portion of the medullary region and the cartilages of the head present certain very interesting relationships which cannot be completely analyzed on the basis of the material available at the present time.

## METHODS

Eggs of Rana pipiens were obtained and inseminated as described by Rugh. Shortly after the completion of the first cleavage twelve typical eggs were selected and placed in a container in a thermostat which held an approximately constant temperature and in which the development took place to the stage desired. The thermostatic assembly was that demonstrated in the laboratory in 1933 and described in a former paper (Hoadley and Brill). A large tank containing a cooling unit was maintained at a temperature of 9° C. In this was placed a smaller aquarium containing a heating element and thermoregulator. The water in the smaller aquarium was stirred constantly by a small motor mounted independent of the tank. The eggs were placed in a container which was suspended in the aquarium. The temperature of the aquarium varied consistently within narrow limits ($\pm 0.25°$ C); that of the water in the vessel containing the eggs maintained a temperature within $\pm 0.05°$ C. Air was blown through the water by means of a medicine dropper at short intervals thus aerating the medium. The temperatures employed will be stated below.

## EXPERIMENTS

Although the larvae which were obtained from the eggs used in the various tests were observed at short intervals during their entire development the description of the individuals may be best considered under three headings according to the species employed and the age of the animals when preserved for histological examination. The first section will deal with preliminary observations made upon Rana sylvatica. The second and third will deal with observations upon larvae of Rana pipiens at the end of 32 hours and 64½ hours of development respectively. The larvae of Rana sylvatica were examined only *in toto;* those of Rana pipiens were also preserved for histological examination.

## RANA SYLVATICA

Eggs of Rana sylvatica were received from ponds west of Cambridge on April 8, 1934. When in the 64 to 128 cell stages they were put in the thermostat at 29.9° C. After 5:40 hours they were advanced blastulae which appeared about to gastrulate. Six hours later (11:40 hours) they still were in the blastula stage though the cells of the vegetative hemisphere were much smaller than those ordinarily found there. They were not as small as those of the animal hemisphere. Twelve hours later (23:40 hours) when eggs held at 21° C were beginning to elevate medullary folds and those held at 18° C were just showing closure of the blastopore, the eggs at 29.9° C still showed no sign of gastrulation, the entire blastula being composed of very small cells even in the vegetative hemisphere. The entire blastula appeared in good condition. At 37:40 hours the condition was very much the same though the cells were even smaller. At 60:50 hours when the experiment was discontinued and the eggs held at 11° C were just showing the closure of the medullary folds, the eggs held at 29.9° C were still in the blastula stage and more than half of them appeared in good condition (fig. 1). The blastulae were very much larger than those of controls at the time of gastrulation. No examination of these blastulae was made to determine the presence or the absence of cilia.

Eggs of Rana sylvatica collected in the neighborhood of Cambridge were brought into the laboratory while in the two-celled stage on March 28, 1935 and immediately placed in the thermostat at 29.95° C. They developed rapidly into advanced blastulae and commenced gastrulation. The yolk plug formed did not decrease markedly in size as gastrulation proceeded but dorsal, lateral, and ventral blastoporic lips appeared while the exposed portion of the vegetative hemisphere remained large. By the 27th hour the medullary folds had closed though the yolk plug still remained open (figs. 2 and 3). Within a few hours the folds at the axial portion of the blastoporic lip (dorsal lip) had closed and a tail bud was evident, being slightly elevated and extending posteriorly. The development of the body and more especially of the head region (fig. 4) was comparable to that of stage 17 to 18 (Pollister and Moore 1937). At the same age other larvae showed an even more extensive development (fig. 5). All of the larvae were very much more advanced than others reared at lower temperatures. By the 36th hour most of the larvae were disintegrating. None was alive

after the fortieth hour. The most interesting feature of the development of these individuals is to be found in the consistent occurrence of the open yolk plug and the development of the tail bud from the median dorsal lip of the open blastopore. Degeneration of the larvae was initiated in the yolky cells of the extruding plug. This subsequently spread to the axial region where it is first indicated by the very irregular appearance of the body ectoderm.

## RANA PIPIENS

Gametes of Rana pipiens were obtained and insemination carried out after the method of Rugh. When the first cleavage was completed in the majority of the eggs, twelve typical individuals were selected and placed in the thermostat. Those to be described below were reared at a temperature of 30.2° C for over 30 hours when they showed extruding yolk plugs, anterior development of head and trunk region, and incipient tail buds slightly more advanced than most of those described above for Rana sylvatica. The larvae were then preserved for subsequent histological examination. All of the eggs raised at this temperature showed the same type of development insofar as the blastoporic region was concerned though they vary slightly in the development of the structures of the head and trunk region. Five cases have been selected for partial description. Transverse, sagittal, and frontal sections were made of larvae. The numbering of the larvae has been altered to enable a continuous series in the descriptions.

*32 hours—#1.*—When examined in toto, this larva (fig. 6) differs from the control in that the frontal region of the head projects slightly more than is usual; the anterior end of the head in the region of the suckers and stomodeal pit is relatively blunt; and the blastopore remains open below the well-formed tail bud. At these higher temperatures the yolk becomes rather brittle and is easily fractured so that unfortunately it is partially lost. This has rendered the general thickening of the blastoporic lips in this case easily visible. Ventrally there is a slight fracture at the base of the tail bud. The axial development of the larva as a whole resembles that of the control. Transverse sections were made of the entire animal.

The sections may best be described in terms of the differentiating components of the larva. The gut will be considered first. The anterior limit of the gut corresponds to that of the control though the archenteric cavity present at the level of the otocysts (fig. 7) is larger and contains a coagulum in which there is yolk material. More posteriorly (figs. 8 and 9) the cavity becomes smaller and the inclusions disappear. The cavity appears progressively more and more ventral in the yolk mass and eventually disappears completely (fig. 10). There is an indefinite suggestion of a smaller dorsal cavity which may be seen in figures 9 and 10. This is entirely lacking at certain levels and where present is filled with a relatively dense yolky material. The brittle nature of the yolk makes good sections hard to obtain and the interpretation is therefore difficult in many sections. Examination of the entire series leads to the impression that in the anterior end the amount of gut is not equal to that of the control in spite of the fact that the anterior limits are comparable. As the blastoporic region is ap-

proached, the yolk-laden endodermal cells are completely segregated within the central cavity and becoming loose project as a more or less disorganized mass in the region of the yolk plug (fig. 11).

The mesoblast of the larvae is plentiful at all levels. This is particularly evident in the somitic regions and in the lateral plate of the trunk (fig. 8). The distinction between the endoderm and mesoderm is not evident in figure 10 but neighboring sections enable one to determine that there also is some hyperplasia in the mesoblast. With the exception of the most ventral portion, the entire margin of the blastopore is lined by a heavy layer of mesoblast which may be seen in figure 11. In that section there is continuity between the external body ectoderm and the inner mesoblast at both lateral lips. More posteriorly in the tail bud itself (fig. 12) the lateral mesoblast bands are typically developed. Anteriorly, in the head region, the mesenchyme is comparable to that of the control.

The neural folds have closed throughout the entire length of the larva. The neural tube shows subordinate differentiation which is grossly similar to that of the control of this stage. Anteriorly the neurocoele is much reduced (fig. 7). More posteriorly it decreases more in size and from the mid-trunk region to the tail bud is either represented by a slit between the two lateral walls (figs. 8 and 10) or is entirely obliterated (fig. 9). The diencephalic region is marked by the attachment of the invaginating optic vesicles which show visual and pigment layers and a patent optic stalk leading to the main portion of the brain. The infundibular sac is present. Posteriorly the spinal cord continues into the tail bud as a solid strand of cells which is easily recognizable in figure 12.

The relations of the vesicular otocysts to the hind brain and the epidermis are similar to those in the control (fig. 7).

The notochord is present throughout the entire trunk region of the larva extending posteriorly to the end of the tail bud (fig. 12) and anteriorly to the posterior margin of the infundibular evagination. Its differentiation is typical though there are local indications of duplication or enlargement (fig. 8). In marked contrast to the situation in older larvae to be described below, the relation between notochord and nerve cord is consistently normal throughout.

The differentiation of the body ectoderm is generally quite typical though there are local thickenings in the form of external papilla-like epithelial growths. The suckers are present (fig. 7) though the mucous secreting epithelium is not as yet deep and contains no secretion. The Primordium of the anterior lobe of the pituitary is well developed and still continuous with the epidermis. The ectoderm over the optic vesicles has thickened as the lens epithelium which, however, has not as yet begun its invagination. Throughout the entire length of the larva the body ectoderm of the dorsal axial region is markedly thinner than that of the flank and belly region. The dorsal fin projection is just forming in the tail bud.

*32 hours—#2.*—This larva, which developed under the same conditions as were described above for #1, showed external features directly comparable to those of the previous case. The case deserves special consideration only in that

the development of the gill plate, of the ventral mesoblast of the blastoporic lip, and of the neurocoele differ slightly from those previously described. Even before the larva was sectioned it was evident that the ventral lip of the blastopore was thicker than that of the more lateral regions. The sections show a heavy ventral mass of mesoblast which was not present in case #1. Similarly the gill plates of the larvae are evident on the lateral head region and in sections these appear associated with lateral expansions of the gut and proliferations of mesenchyme. The neurocoele remains open further posterior than in the previous case. As in case #1, the gut appears to be different in amount in the anterior end though it extends to the same level.

*32 hours—#3.*—The development of this larva differs more from that of numbers 1 and 2 than any of the others considered. The anterior end is definitely arrested in its development and smaller than usual. The blastoporic lips have covered the extruding yolk to a less extent though the ectoderm is sufficient to allow it. The latter is thrown into folds which, in section, are similar to the epidermal papillae. The general picture in most regions is like that of the previous cases. It differs in that the foregut region is more reduced in size and that the development of the optic regions is notably arrested. The two optic primordia appear to be somewhat closer together than in the remaining larvae. It is probable that the departures from the types which are described above are the results of an incipient disintegration which is more evident in the posterior trunk region. The tailbud contains the precursors of the organs found in other larvae though it is not in a healthy condition.

*32 hours—#4.*—In gross appearance, the larva is similar to that described in case #1. The head, trunk and tail bud have developed typically and the yolky plug which extrudes from the open blastopore ventral to the tail bud is intact. It is apparent from the figure of the entire larva (fig. 13) that no extensive involution of mesoblast has taken place at the ventral lip and that the involution along the ventro-lateral lips was not as great as in other cases considered. The larva was sectioned sagitally and the development of the components examined. A section close to the midline (fig. 14) shows the relation between the neural tube, which contains a lumen, and the notochord in the posterior trunk and the tail bud regions. These two structures end in a mass of undifferentiated tissue which lies above the posterior open archenteric cavity. Ventrally, the epidermal epithelium appears to be discontinuous with the thin mesoblastic layer. The slightly disorganized and fractured yolky cells of the endoderm protrude between the latter and the floor of the archenteron. Figure 15 shows a section slightly more lateral in which the place of the nerve cord is taken by the mesoblastic somites. The archenteric cavity is wider in this region than in the midline. Notochord is not present. Posterior to the somites the mesoblast takes the form of a segmental plate which continues to the tip of the tail bud. The slightly lateral ventral lip of the blastopore shows an epidermal epithelium which is continuous internally with the thin mesoblastic layer which underlies the extruding yolky cells. In the anterior regions the larva shows a development similar to that described in case #1.

*32 hours—#5.*—The intact larva (fig. 16) appears to possess anterior structures which have developed to approximately the same extent as those of cases #1 and #4. In the posterior portions the development of the tail bud is not as extensive nor is the extruded yolk covered as completely. The entire larva was sectioned frontally. A study of this case confirmed certain impressions obtained from the examination of transverse and sagittal sections. The notochord is not a straight cord of cells but is bent and gives the impression of being too long for the length of the trunk region. There is ample evidence at nearly all levels that the development of the two sides of the body has been unequal quantitatively and, to a less extent, qualitatively. Figure 17 shows a section cutting the dorsal margin of the open blastopore at which involution of mesoblast has taken place. The epidermal body layer is continuous with the inner segmental plate mesoblast. In the anterior midline the nerve cord containing a neurocoele is present, flanked on both sides by somites which end posteriorly in the aforementioned segmental plate. Passing posteriorly the nerve cord terminates and its place is taken by the notochord for a short space. These fuse in the more dorsal sections but the area of fusion is small. The slight hyperdevelopment of the somitic regions of the right side in the section figured is continued in the sections at other depths. The amount of segmental plate mesoblast varies somewhat but generally appears greater on the left.

## RANA PIPIENS

### $64\frac{1}{2}$ hours

The gametes in these experiments were obtained in the same way as those of Rana pipiens described above. The eggs were inseminated and, after first cleavage was complete, twelve typical individuals were selected and placed in the thermostat at 30.2° C. Development continued and the gastrula was formed as described above. The larvae remained at the high temperature approximately 64 hours when they resembled closely those of stage 21 of Pollister and Moore. The tail with its fins, and, in some cases, head and trunk, were well developed. Over 80% of the eggs used in the experiments were alive and in good condition. All had accomplished gastrulation showing the characteristic open blastopore and the tail bud formed at the dorsal blastoporic lip. The mortality observed was the result of initial local disintegration of the larvae which spread to the surrounding parts, and was not related to the atypical morphogenesis.

"Closure" of the blastopore took place in all but two cases according to the following pattern. The axial elongation of the body continued so that the tail bud projected over and beyond the extruding yolky material. The lips of the blastopore then narrowed and constricted the yolk which was eventually pinched off. Some of the yolk cells formerly located within the larva were then expelled. When the yolk elimination was eventually complete the larval anus appeared. Whether this has developed directly from the open remnant of the blastopore or secondarily in association with a proctodeal invagination cannot be stated. The actual amount of the yolk lost varies slightly in different animals but the method of elimination was the same. The gross development of the trunk

region is similar in all the animals. In one of the cases in which the blastopore remained open after the extrusion of a small amount of yolk, the dorsal tail bud was not large and two secondary posterior extensions appeared at the ventrolateral margins of the blastopore. These secondary protrusions had the gross appearance of tail buds though they were smaller than that of the dorsal margin. Sections, however, show no extensive subordinate differentiation. There is, however, sufficient to make it clear that the dorsal protrusion is a tail bud and that the other two are not differentiated in the same way, but are composed solely of epidermal evaginations containing relatively loose mesenchyme. The anterior end of the larva shows the maximum degree of developmental arrest.

The remaining survivors may be divided into two groups the members of one of which differ from the controls solely in the extent of the development of the gills and the gut. The recovery from the effects of the abnormal gastrulation was nearly complete and took place in 30% of the survivors. The most typical larva appears in figure 18. Such embryos are similar to the controls save that the gut region while open at all levels is consistently smaller and the development of the gills is retarded. This is true to some degree in all cases though the animals differ widely in the amount of reduction. The inhibition appears to effect the development of the gill filaments rather than the primary gill plate.

The remaining 70% of the surviving larvae were defective in the head and trunk regions. The extent of these defects, especially in the more anterior portions, is suggested by the appearance of the larvae in figures 19, 20, 21, 22, and 23. All show various degrees of microcephaly, the reduction being progressively greater in the series as presented. The external appearance of the larvae also indicates that the microcephaly is primarily the result of a reduction of anterior and ventral structures. This is more evident in the sections of the larvae.

Inasmuch as the development attained at this stage conforms rather closely to a general type, the descriptions of the defects will be presented in relation to body regions and organ systems rather than individual cases. The descriptions and illustrations will be based on the five cases mentioned above (figs. 19 to 23) which have been renumbered to give continuity and to emphasize increase in the degree of arrest. Nothing need be said of the larvae which show the recovery from the atypical gastrulation since their development as seen in the sections is very similar to that of the controls. The larvae showing the defects of the anterior region also show progressive effects in the development of the various trunk structures. In so far as possible, the illustrations have been selected to illustrate at least two of the features and hence do not always show the most extreme malformation in any one system.

It was stated above in relation to the development of the notochord of larvae 32 hours old that the notochord was large in many cases and that it showed evidence of having developed more rapidly than surrounding tissues. This has led to bending in certain regions. In the larvae which are 64½ hours old, the extent of the notochord and its differentiation varies greatly. In no case is it present and well developed in the anterior portion of the larva where it showed good development at the earlier stage. It may be rather closely associated with

the gut and composed of a cord of compact cells (figs. 24, 26, 28, 34, and 35) or it may be entirely absent (fig. 44). It may be present in a reduced form at anterior trunk levels, disappear in the median region of the trunk, and reappear in the posterior levels where it continues rather typically into the tail (figs. 25 and 29). The bending of the earlier stage is indicated by the transverse course of the rod in figure 32 and by the reduplication in figure 30. The latter condition does not, however, represent a doubling on itself but a bifurcation. The position of the supporting rod in the dorso-ventral median plane is also quite variable. In many cases it is closely associated with the gut and ventral to the mesoblast of the axial region which surrounds the nerve cord and is differentiating into muscle bands (figs. 24, 26, 27, 28, 32, 33, 35, and 36). In other cases or in other regions it is located in the midline between the somites (figs. 29, 30, and 31). In many cases the notochord may be traced continuously from one position to the other. This will be mentioned below in connection with the development of the muscle masses for it appears to have a relation to the bilateral arrangement of the latter structures. A comparison of figures 7 and 37 is of interest in as much as it will be seen that in the first (32 hours) the notochord is well differentiated for the stage and in the latter ($64\frac{1}{2}$ hours) it is absent. Both of these sections are taken from the level of the otocyst in a region in which notochord might be expected to appear. The condition in figure 37 is characteristic of all of the $64\frac{1}{2}$ hour larvae. In all of the cases the differentiation of the notochord in the tail region is quite similar to that of the control. In no case is the structure in the body region as large as it is in the control.

The mesodermal derivatives may most easily be considered under several headings. The general appearance of the components of the tails is essentially similar to that of the control as may be seen in figure 29 and will not be considered further. The description of the trunk deals first with the axial portion and secondly with the more ventral and lateral regions and the pronephric elements. The head mesoblast presents a condition which is more difficult to analyze and will be considered first.

An examination of the mesoblast of the head region leads to the conclusion that the amount of material present varies rather widely and is more or less closely related to the degree of microcephaly encountered. Thus, while the mesoblast is relatively plentiful in cases 1 and 2 (figs. 37, 38, 42 and 43) and even in 3 (figs. 39, 44, 46, and 47), it is deficient in 4 (fig. 40), and markedly so in 5 (figs. 41 and 45; compare fig. 34). Loose mesenchyme is present in every case in which local condensations of the cells indicate the formation of the pre- and parachordal plates and the precursors of the pharyngeal cartilages (figs. 38, 42, 43, 45, and 46). The amount of mesenchyme around the otocysts varies in the same way and appears to have had some effect on the development of the organ (figs. 37, 40, 41 and 44). The defect in the development of the head mesoblast is even greater than appears in the figures for not only is the amount in the series progressively less in relation to the size of the head region, but the head region itself decreases in size. The amount is probably definitely related to the degree of reduction of the foregut region and the anterior neural crest region

though no direct measurements can be made on these at present. There are vesicles lined by endothelium present surrounded by the mesenchyme. Some of these are isolated and some have united to form channels. Those of the gill region will be considered below in connection with the vacsular system.

The axial mesoblast of the somitic region shows a differentiation which resembles that of the control insofar as the intimate differentiation of the components is concerned but the pattern of the units is markedly divergent. It is true that in many of the sections the dorsal extension of the sclerotomal portion is small or lacking but the myotomal part of the somite is well developed and composed of more or less spindle-shaped differentiating muscle cells. These show a picture slightly different from that of the control in that they are filled with yolk platelets which appear not to have been broken down as they have in the control (figs. 27 and 28). The cells themselves are arranged in bands which appear in a rather disorganized fashion. In some cases they form large bands which lie between the nerve cord and notochord as single masses (figs. 24, 25, 32, 33, 35, and 36), in others they appear as several overlapping masses, some of which are lateral and some of which lie in the midline between the nerve cord and gut (figs. 26, 27, 28, and 34). It will be noted that while notochord is present in most of these cases, it is atypical in its position. Case number 3 shows a rather interesting arrangement of the somitic material which may in part be correlated with the relatively extensive degenerative changes in the dorsal gut region. The axial mesoblast has been displaced dorsally giving the general appearance seen in figures 30 and 31. The notochord is present and lies below the nerve cord in both figures. The nerve cord is nearly solid in both and is actually solid in neighboring sections. In one (fig. 31), there is a dorsal development of the epidermal region which, while it is in no place continuous with the nerve cord suggests an incomplete incorporation of the material of the neural plate. This is the only indication of incomplete closure found in the larvae examined. The somitic material has been shifted dorsally and resembles the distribution of the elements in the cross section of the body region of the lamprey. The mesenchyme of the region varies considerably in the extent of its development (cf. figs. 26, 27, 28, 32, and 33 with figs. 34, 35, and 36). In the fin region (figs. 24 and 25) it appears quite typical. The amount of tissue in which the pronephric tubules are imbedded also varies considerably (cf. figs. 26 and 34) and without a doubt has an influence on the morphology of the tubules. The exact relation is not clear from the material available for study at this time.

The differentiation of the mesoblast lying outside of the axial region appears to be quite specific. Somatic and splanchnic plates are clearly demarked and locally very heavy, especially in the more posterior ventral portion. The continuity between these layers and the more dorsal axial mesoblast is not clear at all levels. There is a subordinate differentiation of blood islands containing numerous blood cells which shows particularly well in figures 32 and 33. The differentiation between splanchnic layer and yolk endoderm is not clearcut at all levels of the trunk. Anteriorly and dorsally in the intermediate cell mass, pronephric tubules are found which, though they appear to be fewer in number

than in the control, nevertheless show a differentiation which is characteristic (figs. 26, 27, 28, 30 and 34). In addition to the tubules of the nephric units themselves, the pronephric duct is present prolonged posteriorly towards the cloaca (figs. 24, 25, 26, 27, 28, 31, 32–36). The posterior extent of the duct is not as great as in the control.

There is a considerable development of vascular elements in addition to the blood islands which have already been mentioned. Heart, aortic arches, and both blood vessels and isolated endothelial vesicles are to be found in every case. The heart shows considerable variation which in a general way follows the degree of suppression found in the anterior regions in the series presented. Thus the organ in case 1 is well developed with endocardium, myocardium and chambers (fig. 37). In 2, however, while myocardium and endocardium are apparently normal, the heart is tubular in shape having few indications of its subdivisions into future chambers (fig. 38). Case 3 (fig. 39) shows a heart which resembles that of case 1. Figure 40 shows the heart of case 4 which is much reduced, the endocardium in the section figured being a solid cord of cells. The pericardial cavity is relatively large. Case 5 has a heart which appears in figure 41 and which, while simpler than that in cases 1 and 3, is more complete than that of the remaining cases and has well developed myocardial and endocardial regions with the indications of the primary chambers. In the first three cases aortic arches are associated with the hearts extending from the anterior conus region towards the gills (figs. 39, 43, and 44). In the remaining two, the arches are present though their course cannot be traced without interruption from heart to gill (fig. 45). Aside from these main vessels others appear at various levels of the body. Some of these are extensive; others are relatively small resembling the vesicles from which the blood vessels themselves develop. Numerous examples of both may be seen in the figures. Blood cells may be seen in some of the larger vessels as well as in the endothelial sacs of the ventral trunk region. They are evident in the larger vessels in figure 38.

The gut region is reduced and much modified in all of the cases as might be expected on the basis of the yolk elimination which has been mentioned above. Anteriorly the foregut region is consistently heavy and solid save in case 1 where a very small lumen is present. In no case is there a patent foregut resembling that of the control and in no case is there a stomodeum present. The pharyngeal pouches are represented by outgrowths of solid masses of endodermal cells which are loose and have no epithelial resemblance. More posteriorly the gut cavity is open and in two cases liver tissue may be identified (fig. 38) with its associated blood channels. The open gut of the anterior trunk region varies greatly in amount and in the size of its lumen (cf. figs. 35 and 36 with 26 and 32) which may be present or absent (fig. 30). The occurrence of yolky material within the cavity of the more anterior trunk gut is not unusual; this is not found posteriorly. Posteriorly the gut region is patent and ends at the anus. In cases 1, 2, and 4 the gut opens to the outside in such a way that it is difficult to state definitely whether or not a proctodeum has been formed, but in case 6 two openings to the outside are found, one of which has no connection with

the gut cavity while the other is continuous with it. Both openings resemble the anus of the control. Two possible explanations are suggested: the first, that there is no proctodeum formed in any of the first cases and that the connection between gut and exterior is through the blastopore which was much delayed in closing, and the second that the persistent blastopore is retained only in the later case in which the junction between proctodeum and hindgut was not established. Certain features lend plausibility to each of these explanations. The degeneration of the endodermal material of the gut region of case 3 which is evident in the figures (30 and 31) has led to constant extrusion of the dissociated yolk cells of that region and hence the closure of the blastopore was not complete in that case. This has not interfered with the typical development of the tail though it is apparent that the area of disintegration was spreading and that the larva was dying.

The nervous system in every case has closed throughout to form either the tube of the control or a solid cord of nervous tissue. In the region of the tail the differentiation is quite typical of that of the control but more anteriorly in the trunk and head regions, the development is definitely arrested. There is constant evidence of subordinate differentiation. This is well illustrated in the hindbrain region (figs. 37, 39, 40, 41 and 44). Nuclei and cell bodies are absent only in the median region of the ventral floor plate. The roof is composed of a thin epithelium which appears wider than in the control. Ganglia are associated with the ventro-lateral walls of the floor. The neurocoele of this region is consistently large in contrast to that of regions immediately anterior and posterior. The appearance in figures 26 and 27 is slightly deceptive for the sections are cut at a considerable frontal angle so that dorsal regions are more anterior than more ventral ones.

Posteriorly in the trunk region the cord shows a retarded subordinate differentiation and a neurocoele which is patent at some levels and entirely lacking in others (fig. 33). The general appearance of the cord is that of a bilaterally organized organ. Ganglia are present though their arrangement appears irregular.

Anterior to the hind brain, the neurocoele becomes much reduced and may persist as a small opening to the very anterior end (figs. 42, 46, and 47) or may disappear leaving a solid cord of nervous tissue (figs. 38, 43, and 45). In all but one of the cases (case 4) a secondary development of what appears to be nervous tissue has taken place anterior and ventral to the anterior tip of the main nerve cord. This may be seen in figures 42, 45 and 46. This cord of cells is entirely independent of the main nerve cord and extends anteriorly into the tip of the larva. In one of the cases it still retains a connection with the epidermal layer at the anterior end. The data available at present do not permit a positive interpretation of either the nature of this structure or its mode of formation. It varies somewhat in size and in shape but is consistent in its general position. In case 4 where this structure is absent the nasal pits are present while in no other case do they appear as definite structures nor is positively identifiable nasal epithelium found. It may be suggested, therefore, that the cord is the re-

sult of the differentiation of a fused and invaginated nasal epithelium which has never developed a patent lumen.

Isolated epidermal sense organs have been found in several cases both in the head region and on the trunk. They are well differentiated.

The eyes are absent or represented by small degenerating areas in all but one of the cases examined. In that case (2), but one of the eyes is present (fig. 48). It has lost its connection with the diencephalon save in one section. The eye appears as an optic cup rather deeply imbedded in the mesenchyme of the lateral head region. The visual retinal precursor is composed of several layers of cells and that of the pigmented retina of a single layer which, however, contains a considerable amount of pigment. At the dorso-lateral margin of the cup (actual dorsal) is a small vesicle composed of a single layer of cuboidal epithelial cells which has been interpreted as a very simple lens vesicle. This latter has no direct connection with the epithelium of the optic cup.

Paired otocysts have developed in all of the larvae. The extent of their differentiation is apparently related to the amount and distribution of the mesenchyme. In some cases the form of the otocysts is comparable to that of the control but in others in which the mesenchyme is not as extensively developed they are more vesicular. Endolymphatic ducts are regularly present (fig. 44) as are also ganglionic masses which are applied to the more ventral median aspect of the ear vesicle (figs. 40 and 41).

The microcephaly characteristic of the head region of the series described above is marked by the approximation, fusion, and in some cases the actual absence of anterior and ventral structures. Some indications of this have already been encountered in the development of the gut and the central nerve cord. In addition there is ample evidence of all three to be found in the development of the nasal placodes and pits, the suckers, and the gills. In general the effects increase with the increase in the degree of general microcephaly but the series is not a perfect one in the case of any one of these organs. Considering the nasal region first we find, as was mentioned above in the section on the nervous elements, that in only one case do they appear (fig. 47) in a form which permits definite identification. As will be seen from the figure the two nasal pits are approximated in this larva until they meet in the midline. In the remainder of the larvae they may be accounted for on the supposition that they have fused in the midline and subsequently have invaginated as solid cords of nervous tissue. This conclusion is strengthened by the observation that in one case the anterior solid nervous cord is continuous with the epidermis.

The suckers of the larvae examined are not paired in any of the cases but appear, with one exception (in which they are entirely lacking) as median sucker plates usually located at the most anterior tip of the head (fig. 46). The subordinate differentiation of the sucker itself is similar to that of the cells of the typical organ. The defect is, therefore, one of size and of location. It may be repeated here that the stomodeum is not found in any of the cases.

The extent of gill formation has already been discussed briefly in connection with the gross appearance of the larvae which are described here. The location

of the gills on the body is best appreciated in the transverse sections where their ventral position is evident. The approximation may be explained in terms of ventral migration or in terms of suppression of ventral structures. The latter view is probably the correct one. While gills may be seen in many of the sections their ventral position is most marked in case 3 (fig. 44) and case 5 (fig. 45). It will be recalled that the microcephaly was most extreme in 5. The suppression of the number of filaments formed in each gill region is shown in several of the figures but is seen best in the figures of the entire animals.

One further observation deserved mention though the treatment of the material has not been such as to yield conclusive information as to the structures involved. In several cases cells which resemble both the infundibulum and the hypophysis have been found in the sections associated with other structures which have been used as aids in their identification. The tissues are less extensive than in the control. The occurrence of these organs might be anticipated for both appeared in material treated in the same way and sectioned after 32 hours of development. On the other hand other organs present at the earlier stage have either degenerated or have failed to develop in the larvae used here.

## DISCUSSION

Attention has already been called to the fact that when eggs are placed at the high temperature immediately after the first cleavage their behavior is different from that of eggs transfered at the 128-cell stage. The results point to a more complete adjustment to the new conditions on the part of the first group. Preliminary experiments on the transfer of early gastrulae and very late blastulae indicate that such stages initiate gastrulation but are unable to complete the process and the result is death. Transfer in early blastula stages results in the formation of giant blastulae which never undergo gastrulation. The forms are viable and show very small cells in both the animal and vegetative hemispheres. Similar forms have been described by Dawson ('38) in which differentiation of cilia has taken place. The usual morphogenetic differentiations associated with gastrulation are absent. The experiments cited by Dawson differ from those described here in that such giant blastulae were obtained from eggs which were placed in 2,4-dinitrophenol while in the 2 cell stage as well as in the early blastula. While no cilia were actually seen in the above preparations it may be assumed that they were present as in the cases described by Dawson. Information as to the extent of the ciliation would also be of interest. The results recall those obtained by Lillie ('02) with the egg of Chaetopterus. The critical nature of the period of gastrulation is indicated by the death which follows in cases where the process is initiated but not completed. In all of the animals which were placed in the thermostat at the high temperature immediately following the completion of the first cleavage plane, gastrulation took place and a persistent yolk plug was formed. Subsequently there was a "closure" of the yolk plug which, in a few instances was followed by the production of an essentially complete larva comparable to the control.

Persistent yolk plugs have been obtained in amphibian eggs by a number of

methods. But few methods have yielded results closely resembling those described above both as to structure and subsequent development (Hertwig '94, and '95; Holtfreter '33a and b; Dawson '38). Lillie and Knowlton ('97), discussing the behavior of animal and vegetative hemispheres of the amphibian egg as affected by extremes of temperature, have called attention to the fact that high temperatures accelerate the development of the animal hemisphere more than the vegetative, and that low temperatures inhibit the development of the vegetative hemisphere more than the animal. There is a similarity in the gross morphological pictures resulting from the two differential effects. Both have been shown by Hertwig and by Schultze ('94) to result in open blastopores. On closer examination, however, the two cases appear quite different. At low temperatures the medullary plate is formed but does not usually close completely (compare effects with various salts, etc. Holtfreter '33; Piiper '33; Morgan and Tsuda '94; Hertwig '95; Cohen '38). Moreover, the mesoblast and neural material differentiate while still in the margins of the open blastopore leading to spina bifida. Such results might seem to furnish conclusive evidence that concrescence plays a fundamental role in the morphological development of the axial portion of the embryo. The results obtained with the high temperature and with 2,4-dinitrophenol, however, present a very different picture. In none of the temperature experiments did the neural plate fail to close. The margin of the blastopore never showed a differentiation of its lips in terms of half embryos, and the tail bud which developed from the dorsal margin of the open yolk plug stage (Hertwig has compared this to the typical appearance in the Elasmobranch) contains large plates of mesoblast which resemble those found in the primitive streak and segmental plate region of the bird blastoderm. Completely formed neural plates in the 2,4-dinitrophenol experiments also always closed. When the two conditions are compared, therefore, it would seem that the most satisfactory explanation is to be had by assuming that temperature has a differential effect on the processes involved in morphogenetic development of the germ and those concerned in histogenesis and embryonic segregation. At the low temperatures the morphogenetic processes are more retarded than those related to segregation and hence the differentiation of the

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FIGURES

Figures 1 to 5, Rana sylvatica; Figures 6 to 48, Rana pipiens
Figures 6 to 17, 32 hours at 30.2° C; Figures 18 to 48, 64½ hours at 30.2° C
For explanations see text.

PLATE I

Fig. 1. Permanent blastula. At 29.9° C from 64–128 cell stage to 60:50 hours.
Fig. 2. Open blastopore; incipient tail bud. At 29.95° C from 2-cell stage to 27+ hours.
Fig. 3. Same treatment as Larva in Fig. 2.
Fig. 4. Same treatment as Larvae in Figs. 2 and 3.
Fig. 5. Same treatment as Larvae in Figs. 2, 3, and 4. Yolk plug removed.
Fig. 6. Open blastopore (partially removed) and tail bud; Leica photograph.
Figs. 7 to 12. Transverse sections from Larva 1 (Fig. 6).
Fig. 13. Open blastopore and tail bud; Leica photograph.
Figs. 14 and 15. Sagittal sections from Larva 4 (Fig. 13).
Fig. 16. Open blastopore and incipient tail bud; Leica photograph.

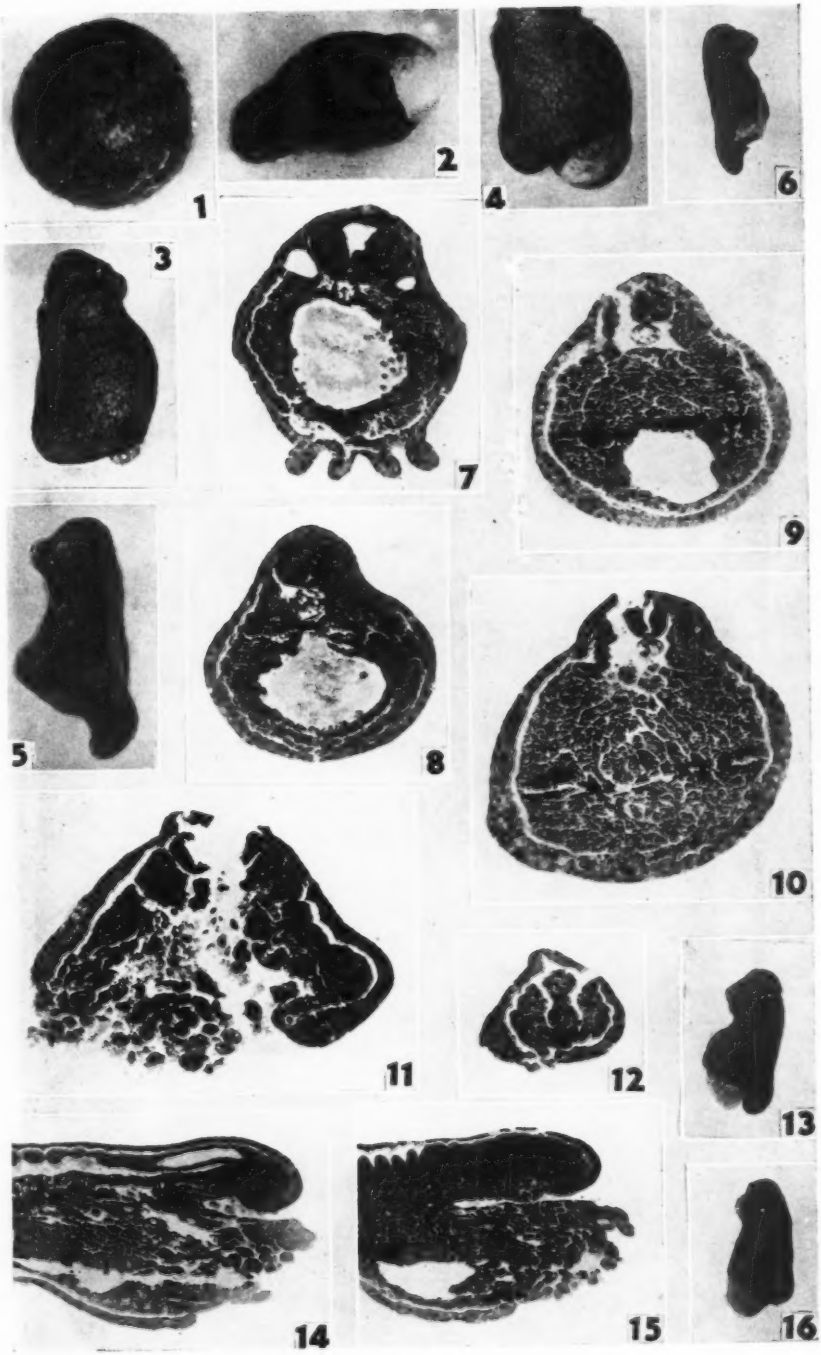


PLATE I

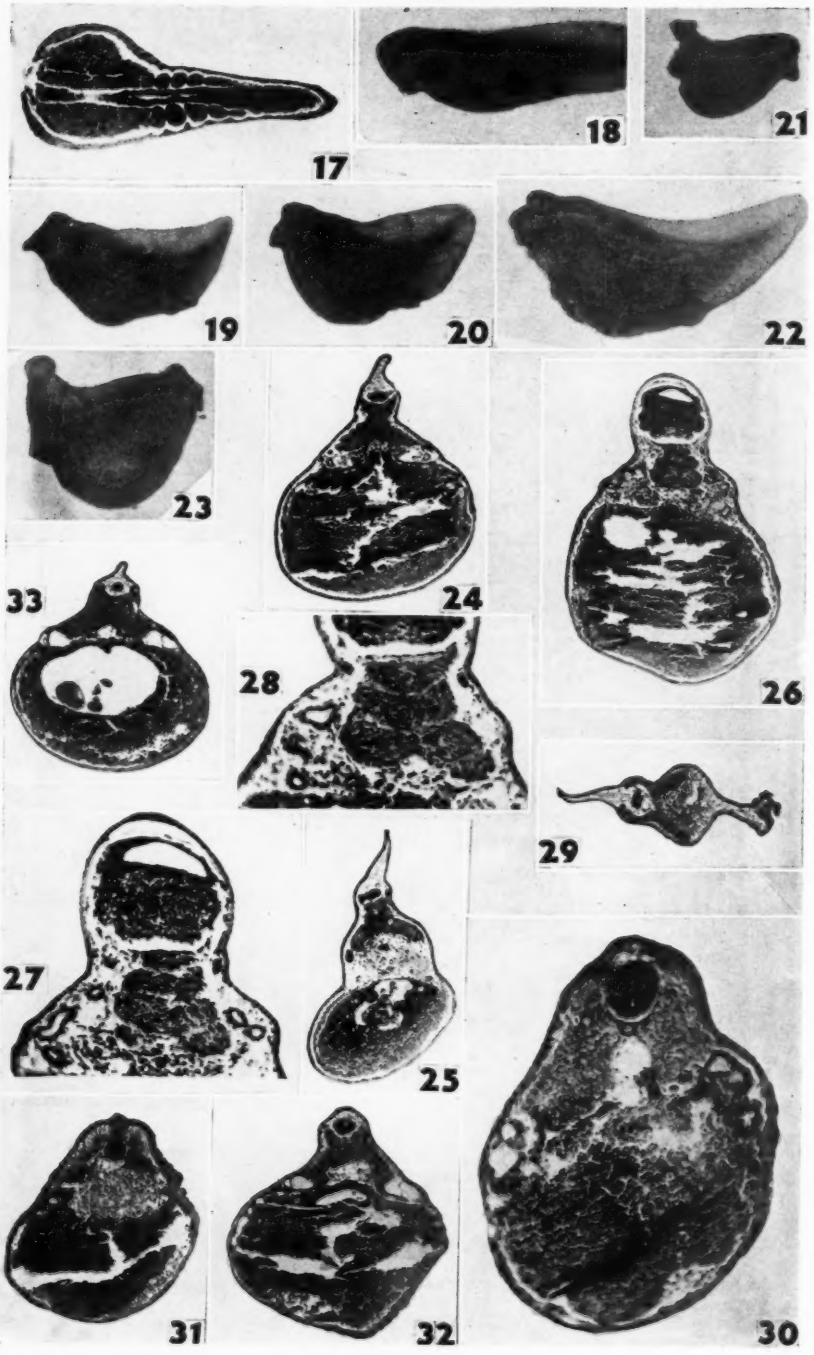


PLATE II

lateral body regions takes place before they reach the axial position. The elements responsible for the inductive processes, which in turn are concerned in segregations, are already present and in effective relations, evocator to responding system. As might be expected, subsequent morphological adjustments are in a large measure impossible. At the high temperature, the morphogenetic processes are accelerated to a greater extent than are the histogenetic and hence the cell migrations and the gross morphological rearrangements within the embryo precede the period of embryonic segregation and evocation. Over by far the greater part of the temperature range the disjunction between the two processes is not sufficient to produce visible evidence of differential retardation or acceleration. There is no conflict between the assumption that there is a differential action of temperature in histogenetic and morphogenetic processes and the fact that the two extremes affect the relative rate of cleavage in animal and vegetative hemispheres of the egg in the same way.

The eventual closure of the blastopore takes place by a combination of two processes, growth on the part of the lips of the blastopore and the extrusion of part of the yolk-laden cells of the archenteron. The process bears certain superficial resemblances to the encircling of the yolk by the blastoderm in certain fishes (toadfish) but should be regarded rather as a completion process. There are certain resemblances to the morphological changes encountered during post-generation and regeneration. It is interesting to note that in respect to the growth in length which accompanies the early development of the embryonic axis, tissues exhibit a differential response to temperature. Thus the notochord is longer than would be expected and bends on itself. This bending is not accompanied by a similar bending in the nerve cord or displacement of the myotomal material. The development of the notochord is typical in the more posterior levels of the axis, though anteriorly the isolation of the structure from the dorsal archenteric roof may be incomplete or wanting. Larvae lacking notochord have also been obtained by Lehmann by means of LiCl ('34 and other references below). Both incomplete isolation of notochord and its absence are associated with unusual conditions in the somites, more specifically in the region of the myotomes.

Median transverse myotomes have been reported at least four times. Lehmann ('37a and b) and Cohen ('38) have obtained "fusion" of the myotomes of the two sides of the body following treatment of amphibian embryos with LiCl, and Lehmann ('28) and Hoadley ('28) have obtained similar results after removal of axial structures from the amphibian embryo and the fish embryo respectively. In the latter type of experiment the notochord was removed and

PLATE II

Fig. 17. Frontal section from Larva 5 (Fig. 16).
Fig. 18. Larva showing extensive recovery; typical save for gill.
Figs. 19 to 23. Larvae showing progressive effects; note gills and microcephaly.
Figs. 24 and 25. Transverse sections from Larva 1 (Fig. 19).
Figs. 26 to 29. Transverse sections from Larva 2 (Fig. 20).
Figs. 30 and 31. Transverse sections from Larva 3 (Fig. 21).
Figs. 32 and 33. Transverse sections from Larva 4 (Fig. 22).

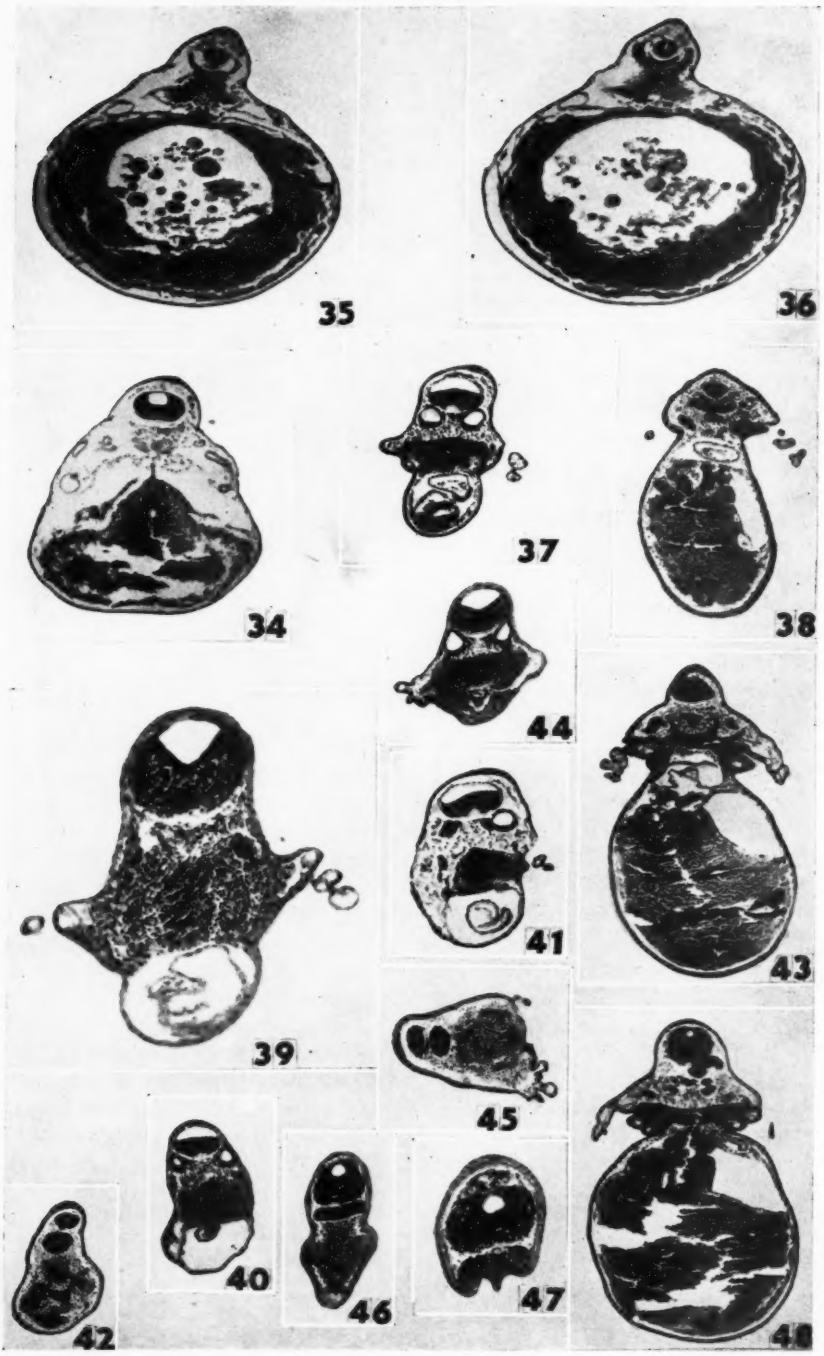


PLATE III

absent (amphibian) or both notochordal and neural precursors were removed and absent (fish). In the experiments with Lithium and in the temperature experiments reported here, the development of the notochord was either completely or partially arrested. The interpretations given by Lehmann and by Cohen to the results following exposure to Lithium differ. Lehmann favors an explanation which assumes mesodermalization of the notochordal material and the incorporation of the cells in the central myotomal mass; Cohen favors an explanation in terms of non-segregation of the notochord and hence an "endodermalization" of the notochordal precursor. The general appearance of the material available for study in the present series of experiments confirms Cohen's interpretation though it would not appear necessary to postulate either an endodermalization or a mesodermalization. The actual displacement of the notochord alone, as pointed out by Cohen, results in a subsequent fusion of the myotomal precursors. The general relationships are not as clear in the present experiments as they were in those on the fish cited above, but the position taken by the myotomal mass is the same.

Not only is the notochord formation arrested in the anterior end of the larva but the development of the gut itself departs widely from that in the control. In place of the open foregut with its pharyngeal pouches one finds a solid mass of endodermal cells; the pouches are also solid; both are reduced in extent. The only specific histological differentiation is found in the liver. Associated with these defects in the development of the gut is a deficiency in the head mesoblast. The mesenchyme which is present is differentiating in a more or less typical fashion but it is quantitatively inadequate. This is very significant in view of the microcephaly which will be considered below. Similar defects are occasionally found in the trunk region though the general appearance posterior to the gills is much more typical. The wide and open gut, containing fragments of yolky material, has been mentioned by Morgan and Tsuda, and by Holtfreter. Morgan and Tsuda state that "The most noticeable structure is the large archenteron . . ." and Holtfreter remarks that the material within the cavity is found in those regions where it is found in the control. Both of these observations have been confirmed in the present experiments. In the posterior trunk region the cloacal portions of the endodermal tube are quite similar to those of the control. In one case there is evidence that the entire cloaca and the anus are formed from the persistent blastopore. In other cases their derivation is not known. It would appear probable that anus and cloaca may develop either with or without the usual contributions from the proctodeum. The presence of an independent proctodeal invagination is not alone sufficient evidence for the derivation of the definitive ectodermal portions of the cloaca and anus from the closing blastopore for it is conceivable that in some cases the proctodeum itself may be duplicated.

PLATE III

Figs. 34 to 36, 41 and 45. Transverse sections from Larva 5 (Fig. 23).
Figs. 37 and 42. Transverse sections from Larva 1.
Figs. 38, 43 and 48. Transverse sections from Larva 2.
Figs. 39, 44, 46 and 47. Transverse sections from Larva 3.
Fig. 40. Transverse section from Larva 4.

It is quite apparent from the examination of both the 32 hour and the 64½ hour material that the yolk is not utilized to the same extent in the experimental animals as in the controls. The presence of large numbers of yolk plates in the cells, especially in the elongating cells of the myotomes is usual in the experimental animals; the plates are rare in the cells of the control larvae. This would indicate that the high temperature has retarded the rate of yolk utilization and is further evidence of the independence of the histogenetic processes.

The microcephaly which is so evident in all of the cases may be explained, in part at least, on the basis of the suppression of the development of the foregut. As has been mentioned above, the anterior extension of the foregut is not equal to that of the control in any of the experimental animals which exhibit defective development. A similar microcephaly has been obtained by Töndury ('37) and related by him to the defective development of the foregut following the reversal of its precursor in Triton alpestris. In the pharyngeal region the larvae consistently show reduction in both gills and heart. Anteriorly the microcephaly is accompanied by partial or complete arrest of the epidermal derivatives comprising the facial region. In none of the experimental cases showing defects did the stomodeal invagination appear. This might be expected in view of the absence of the foregut in that region. The adhesive area, which has a fairly wide precursor in the frog (Halter '36, fig. IIIB) is reduced to a small round organ that is consistently terminal in position. The facial field is much reduced and the more dorsal band in which the precursors of hypophysis and nasal placodes are located has been condensed in the midline so that the hypophysis is small and the olfactory placodes are fused and remain external in but one case. The approximation of the organs is most evident in the anterior and ventral regions. In no case did it involve structures posterior to the level of the pharynx. Dorsally there is a developmental arrest in the neural tube. Little subordinate differentiation is to be found anteriorly though the myelencephalon is indicated by its thin roof and the presence of some differentiation in the ventral floor plate. The eyes are completely lacking save in one case where one eye is found deeply imbedded near the brain. This condition resembles that described by others after various treatments. Both otocysts are consistently present and show some subordinate differentiation and the associated ganglia.

The above discussion is based entirely upon the larvae which displayed definite defects of the anterior region. While all of the eggs which developed at the high temperature showed the delayed closure of the blastopore, approximately 30% eventually recovered from the effects so completely that they differ from the controls only in the development of the gills. On the basis of gill development a series of larvae may be made in which the most complete is approximately normal and the least complete shows very extensive suppression of the filaments. This series, however, does not parallel that of anterior developmental arrest and hence is of little use in explaining the normal appearance of one-third of the larvae and the graded microcephaly in the remainder. The development of the foregut is a much better indication of the development which has been attained in other parts. In this series progressive arrests are found in most of

the other systems. The heart and aortic arches are the chief exception. The larvae which approach the controls in their development show none of the outstanding defects which have been mentioned in the sections above save for the failure of yolk utilization. There is some variation in the development of organ structures other than gills but these are no greater than are ordinarily encountered between normal larvae.

In the present investigation, the series of effects obtained is the result of individual variations in the responses of the larvae. Only one temperature was employed and all of the transfers were made at one stage in development, viz., after the completion of the first cleavage plane. It may be assumed that there would be a closer correspondence between degree of effect and temperature if a more complete analysis were made, with a number of temperatures in the neighborhood of 30° C and a number of transfers made at different stages of development. A correlation of these results with those obtained by transplantation methods and studies on prospective significance may be expected to lead to a more exact knowledge of the processes involved. Such a study is now being made.

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# NEURAL INDUCTION BY MEANS OF INORGANIC IMPLANTATION

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*(Received February 2, 1938)*

## PROCEDURE

Substances to be tested for inductive properties were introduced by means of sharp forceps into the blastocoele of blastulae or early gastrulae of the red-belly newt, *Triturus pyrrhogaster* (Boie), which is the most common in this district.

Following implantation each embryo was raised separately on silk cloth in a small glass vessel filled with ordinary fresh water until some recognizable change occurred in the germ. Daily observations were made and records were kept, supplemented by sketches where necessary. After a specified period of time had elapsed, the embryos were fixed in Smith's fluid. Histological changes of the ectoderm and the cells in the interior of the embryo were studied in sections stained with gentian violet.

## INTERPRETATION OF THE RESULTS

Although but three substances, with differing properties, were employed in this series of experiments, namely fuller's earth, silica and calcium carbonate, the results obtained appear to demonstrate that the induction of neural plate in the Urodele may take place following the introduction of an inorganic substance into the blastocoel.

At the beginning of this investigation, fuller's earth was used as the carrier of inductive agents merely as a modification of the method of Barth (1934), who used kaolin in its place. It was not suspected at that time that neural induction might follow the introduction of such a mineral material alone. Barth described the secondary neural plate he obtained in *Amblystoma* embryos as having been provoked by the substances which were mixed into kaolin. However, the question is raised here as to whether, in reality, the induction was due, as he contends, to the particular effect of the mixed substances. It is most regrettable that control experiments with the use of kaolin alone were not made.

The properties of fuller's earth used in experiments vary according to the source and, if obtained from the same source, according to depth from which it originates. It may be acid, alkaline or neutral in the litmus reaction. The induction tests, however, yield positive results, though in slightly different degrees, whether the earth is acid or alkaline. It is at least evident that the stimulus for neural induction is not related to the hydrogen-ion concentration of the substances concerned. From the additional fact that the induction test likewise proves positive when fuller's earth is neutral, it follows that if the neural plate is regarded as coming into existence under the direct influence of the introduced material, the inductive power cannot be related either to an acidity

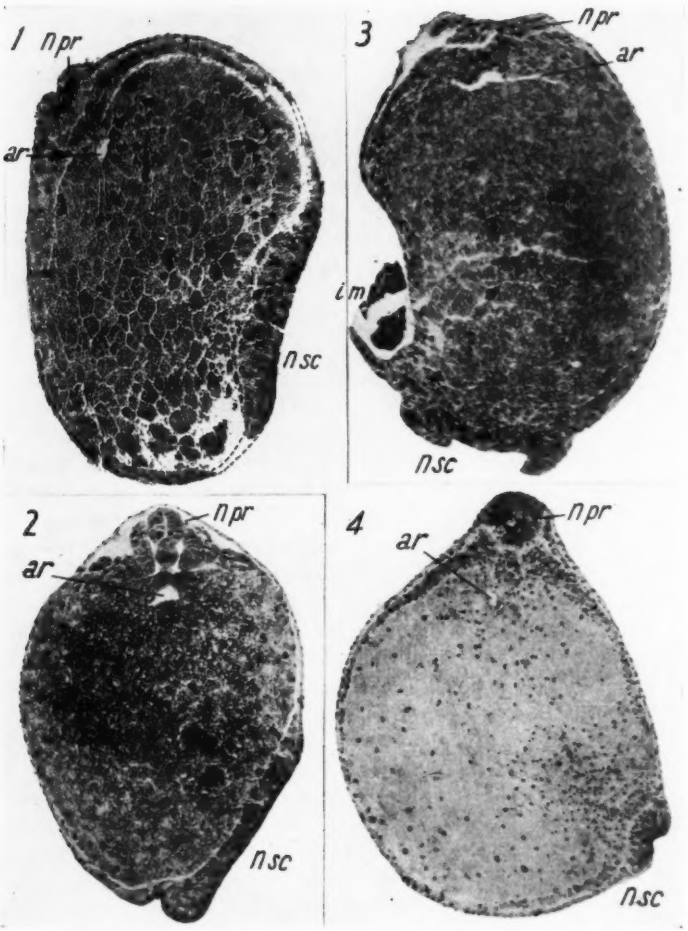
or alkalinity, but must be associated with other characteristics of the substances employed. (Compare the results of Fischer, C. S., 1935.) It was also established in the same series of experiments that induction of a neural plate is possible through the introduction of $SiO_2$ and $CaCO_3$, both of which are practically insoluble in water and have nothing common in their chemical constitution. Hence, it must be seriously questioned whether neural induction in these cases was really a result of the direct influence of the material introduced.

If the inorganic substances named are placed within the blastocoele, as when organic materials are employed, the formation of neural plate always occurs at or near the point of contact of the ectoderm with the introduced object. If it lies deep in the interior of an embryo, no modifications are recognizable even in that region of the ectoderm nearest to it. But there are two noteworthy exceptions: first, even though the introduced material remains at a considerable distance from the surface, if a marked disintegration of the tissue around it occurs and if a stream of the products of this disintegration reaches the ectodermal area, an ectodermal thickening is formed there, a kind of nervous organization resembling the neural plate being sometimes produced (figs. 1 and 2). Second, the neural induction was also brought about when the introduced material did not give rise to a cellular disintegration inside of an embryo, but rather apparently resulted in one of the following situations: (a) a part of the archenteron was displaced and had an abnormal position, or (b) a histological structure comparable, at least, to the dorsal wall of the archenteron was differentiated (figs. 3 and 4). The closure of the neural plate into a canal is especially marked in these instances (fig. 4). Hence in the latter series of experimental cases, it is reasonable to conclude that the induction phenomena are due not to the direct influence of the material introduced, but rather to the abnormalities—especially those affecting the structure of the archenteron—provoked in an embryo by the introduction of a foreign substance. From these considerations it is evident that the induction process at work here consists, in principle, of nothing more than the mechanism normally involved in the formation of a neural plate through the contact of the dorsal wall of the archenteron. Regarding the case first mentioned of the ectoderm coming into contact with the disintegration products of the internal tissue, it must be borne in mind that the endoderm which, when alive, does not possess the power of induction, was shown in some of Holtfreter's experiments (1934) to exert a positive effect in induction tests after it had been killed. Hence, it may be permissible to presume that the endodermal cells also acquire a similar power of neural induction when they disintegrate. Moreover, in those specimens in which the implant lies in immediate contact with the ectoderm, more or less disintegration of the endodermal tissue is often found to have taken place at the point of contact. Since the formation of a supernumerary neural plate was considered to be due to the products of cellular disintegration in the foregoing cases in which the introduced material remained inside of an embryo, no contradiction will result, if we assume the induction here to be the result of the disintegration products of the tissue at the place of contact. From this point of view what is, then, the part played in the induction phe-

nomenon by the inorganic substances used here? Is it possible that the implant has any effect other than a mechanical one? The neural induction which follows the introduction of inorganic substances, as shown in the above experiments, may be explained on the basis of a direct mechanical stimulation of the ectoderm. It is more probable, however, that the very process of implantation leads first to a disintegration of the internal tissues, the products of which subsequently exert an influence on the ectoderm at the time most favorable for the development of neural plate, the induction thus being brought about in an indirect manner.

From the view-point that the formation of a neural plate is induced indirectly through mechanical stimuli, how may the failure of previous experiments in which starch, bread, yeast, agar, gelatine, lard, wax, coagulated egg albumen, blood of hen, powdered charcoal, etc., were introduced, be explained? The answer may be rather simple. So long as these substances themselves do not have a specific chemical capacity to act as stimuli to cells, it may be supposed that their physical properties are not such as to cause disintegration of cells in the interior of an embryo. But we must not conclude from the foregoing results that the implantation of pieces of various kinds of tissues derived from widely different kinds of animals, conducted by Holtfreter and others, results in a situation similar to the above, the implant playing an indirect role in neural induction. It can hardly be doubted that some of them take a direct part in the formation of a neural plate from the ectoderm as these investigators state. Their interpretation of the results, viz., that all the materials used have a similar inductive effect on the formation of a neural plate, cannot be recognized as valid without further examination.

It has been mentioned above that living embryonal tissues, from the same species as the host, have no inductive capacity, while the same tissues, when killed by heat or with chemicals, acquired a capacity for neural induction. Whether this is due, as Spemann (1934) supposes, to the presence of an inhibitory substance in addition to the inductive one, or to the fact that the cell is more permeable to the latter when tissues are killed (Holtfreter 1934), we have at present no opinion to offer. It may be supposed that the introduction of the embryonal tissue of the same species does the least harm to the host embryo, and is the least likely to initiate cellular disintegration. However, a piece of tissue once boiled or treated with chemicals is to be regarded as nothing but a sort of organic material. Whether or not this can remain harmless to the host embryo as in the living condition is an open question. In the case where an inducing substance is contained in the implant, the formation of a supernumerary neural plate is obviously to be attributed to the effect of the implant itself. In the case where the graft is not possessed of such a potency, if an autolysis or necrosis on the part of the host tissue is brought about by its presence, the graft may be said to be the cause, though indirect, of the induction of a secondary neural plate, as is the case with the introduction of inorganic substances. It seems to be somewhat hasty to conclude, therefore, that the various kinds of animal tissues and organic materials which, being brought into the blastocoele




1. Neural induction in *Triturus*-embryo by fuller's earth kneaded with Sörensen's buffer solution pH 8.0; Fixed about 4 days after operation.

2. Showing the same induction as before through the introduction of neural fuller's earth alone into the blastocoele of an early gastrula; Operated on May 30th and fixed on June 3rd.

3. Another example of the neural induction through fuller's earth with alkaline reaction to the litmus; Operated on May 24th and fixed after 3 days, i.e., on May 27th. In this specimen a structure comparable to the dorsal wall of the archenteron is called into differentiation just beneath the induced neural plate.

4. Neural induction through the introduction of $SiO_2$; Operated on May 20th and fixed on May 24th. Here the neural plate is almost closed into a tube.

All figures are reproduced 33 times as large as the original size. The inorganic organizer is not shown except in one case (fig. 3) in which the introduced material is extruded out of the embryo and is enclosed by the ectodermal flap of the damaged portion. Although no particular thickening can be recognized in the ectoderm which is in immediate contact with the material, somewhat apart from this position, on the ventral side of the embryo, a neural plate nearly of typical structure is found to be formed. In other specimens the implant is not shown in the figures, but it is found to be contained in the embryos several sections away from the ectodermal thickening.

Lettering common to all figures: *ar*, archenteron, *im*, implant, *n pr*, primary neural plate or tube, *n sc*, induced plate or tube.

of an embryo, are instrumental in inducing the formation of a neural plate, are all provided with the inductive power and are, hence, in possession of an inductive substance which is common to all. What is needed here is to inquire first of all in what manner the tissues play their part in the process of neural induction.

## SUMMARY AND CONCLUSIONS

The inductive development of a neural tissue or even plate is realized in the embryos of Urodelan *Triturus pyrrhogaster* through the introduction of mineral materials, such as fuller's earth, silica and calcium carbonate. It is probable that the induction by inorganic substances is a mechanical effect. It is conceived that the very process of implantation leads a disintegration of the internal tissue, the products of which then exert an influence on the ectoderm at the time most favorable for the development of the neural tissue, the induction thus being brought about in an indirect fashion.

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# DEVELOPMENTAL GROWTH AND NUCLEIC ACID COMPONENTS VI. ADENINE

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This report begins the survey of the purine components of nucleic acid with respect to their influence singly on growth of *Obelia geniculata*.

Details of description and procedure are referred to elsewhere (Hammett and Schlumberger '37). The adenine used here was the sulfate; prepared, identified, and purified by Schuchardt. Stock solutions containing 0.101 gram in 170 cc fresh sea-water with addition of circa 4.0 cc N/10 NaOH for neutralization provided daily the aliquots from which test culture solutions were made.

Sixty-nine experiments were run with close to 34,000 animals, half of which were exposed to the compound in concentrations ranging from M/200,000 to M/6250. Tests and controls were run simultaneously under like conditions of temperature, illumination, and pH. Four changes into fresh sea-water were made during the 24 hour course of each experiment. The data are given in Table 1. The method of its derivation, construction, and analysis are found in the report by Hammett and Schlumberger.

TABLE 1

TREND OF TEST DEVIATION FROM CONTROL WITH RESPECT TO DEVELOPMENTAL AND METABOLIC ACTIVITY IN ADENINE CULTURES. ALSO THE NUMBER OF COLONIES, HYDRANTHS, AND GONOPHORES AT THE STATED CONCENTRATIONS

| M/1000 | 200 | | 100* | | 100 | | 50 | | 25 | | 12.5 | | 6.25 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| No. Expts. | 10 | | 9 | | 10 | | 10 | | 10 | | 10 | | 10 | |
| | Cont. | Test | Cont. | Test | Cont. | Test | Cont. | Test | Cont. | Test | Cont. | Test | Cont. | Test |
| No. Colonies | 150 | 150 | 135 | 135 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 |
| No. Hydranths | 2515 | 2510 | 2233 | 2232 | 2454 | 2481 | 2440 | 2470 | 2363 | 2372 | 2499 | 2493 | 2455 | 2468 |
| No. Gonophores | 151 | 177 | 89 | 81 | 262 | 264 | 216 | 224 | 203 | 252 | 319 | 337 | 223 | 226 |
| | GROWTH | | | | | | | | | | | | | |
| | + | − | = | + | − | = | + | − | = | + | − | = | + | − | = | + | − | = | + | − | = | + | − | = |
| *Initiation* | 1 | 2 | 4 | 1 | 3 | 3 | 1 | 4 | 2 | 2 | 2 | 3 | 2 | 3 | 2 | 1 | 2 | 4 | 0 | 4 | 3 |
| *Proliferation* | 3 | 3 | 2 | 2 | 2 | 4 | 0 | 4 | 4 | 3 | 2 | 3 | 2 | 3 | 3 | 2 | 4 | 2 | 2 | 4 | 1 |
| *Differentiation* | 3 | 3 | 4 | 2 | 5 | 3 | 4 | 4 | 2 | 7 | 2 | 1 | 4 | 3 | 3 | 4 | 2 | 4 | 3 | 5 | 2 |
| *Organization* | 5 | 2 | 2 | 3 | 2 | 4 | 4 | 2 | 2 | 3 | 3 | 3 | 2 | 2 | 5 | 3 | 2 | 4 | 4 | 2 | 3 |
| | METABOLISM (By Single Experiment) | | | | | | | | | | | | | |
| *Maintenance* | 5 | 4 | 1 | 3 | 5 | 1 | 3 | 4 | 3 | 6 | 3 | 1 | 5 | 3 | 2 | 6 | 3 | 1 | 4 | 3 | 3 |
| *Catabolism* | 4 | 4 | 2 | 3 | 4 | 2 | 4 | 5 | 0 | 8 | 2 | 0 | 8 | 2 | 0 | 2 | 5 | 3 | 4 | 5 | 1 |
| | METABOLISM (By Combined Experiments) | | | | | | | | | | | | | |
| | Cont. | Test | Cont. | Test | Cont. | Test | Cont. | Test | Cont. | Test | Cont. | Test | Cont. | Test |
| *Regression* | 40.6 | 41.4 | 38.1 | 39.0 | 22.2 | 24.4 | 35.7 | 34.0 | 30.2 | 28.6 | 30.0 | 27.7 | 38.2 | 34.1 |
| *Catabolism* | 80.4 | 81.1 | 77.7 | 75.9 | 83.3 | 83.4 | 80.1 | 84.8 | 70.0 | 73.7 | 90.1 | 88.5 | 77.3 | 76.8 |
| | *Increase in Percentage Regression* | | | | | | | | | | | | | |
| | 1.9 | | 2.4 | | 9.0 | | −4.8 | | −5.3 | | −7.7 | | −10.7 | |

* 1936 Data; all the rest are 1937.

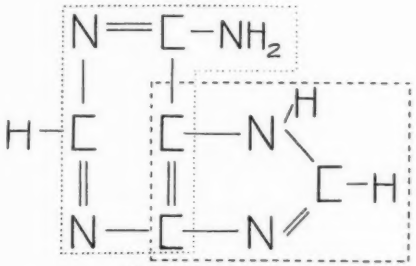
## RESULTS

Adenine is slightly toxic to obelia growth at M/6250. The evidence is that of the 34 growth expressions 15 were less in test than control and but 9 were greater; while at M/12,500 test growth was less than control in 10, greater in 10, and equal in 14. A shift to retardation of catabolic expression is supportive. This began at M/12,500. However, since test growth was as good as control at this level, the data thereof will be used as well as those from M/200,000 to M/25,000.

Changes expressive of metabolic reaction were definite though small. Maintenance may have been cut a bit at M/100,000; but from then on it was sustained with increasing intensity and test regression became more and more less than control. There was no evidence of specific dynamic effect unless the reaction at the low M/200,000–M/100,000 range be so taken. The variations in catabolic expression allow no present interpretation.

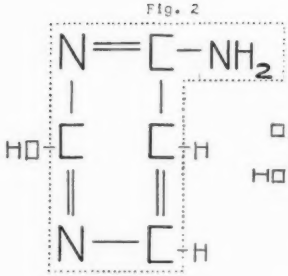
Initiation was retarded in the presence of adenine. Thus—new organism production in the tests was less than control in 16 and greater in but 8 of the 42 expressions of this activity given by the 6 sets of experiments. By concentrations it tended to be less in 4 and greater in none of the 5. And when the data are combined test was less than control in 5 and greater in but 1 of the 7 potentialities for activity expression.

Fig. 1



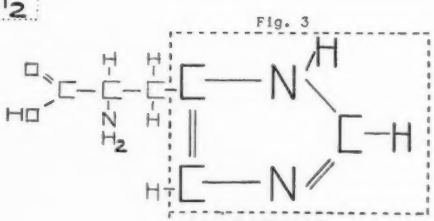
ADENINE

Fig. 2



CYTOSINE (enol)

Fig. 3



HISTIDINE

PLATE I

Test proliferation may have been held back. Thus—it was less than control in 18 and greater in but 12 of the 48 expressions given by the six sets of experiments. By concentrations it tended to be greater in 1 and less in 2 of the 5. And when the data are combined test was less in 5 and greater in but 3 of the 8 potentialities. The extent of deviation was slight.

Test differentiation may have been advanced. Thus—it was greater than control in 25 and less in but 19 of the 60 expressions given by the 6 sets of experiments. By concentrations it tended to be greater in 2 and less in 1 of the 5. And when the data are combined it was greater in 4 and less in 3 of the 10 potentialities. Here too the deviation was slight.

Organization, however, was definitely enhanced. Thus—test expression of this activity was greater than control in 19 and less in but 13 of the 54 opportunities given by the 6 sets of experiments. By concentrations it tended to be greater in 3 and less in none of the 5. And when the data are combined it was greater in 5 and less in but 2 of the 9 potentialities. Furthermore the reaction extended into the toxic M/6250 zone.

## DISCUSSION

Analysis of much control data brings out a positive correlation between percentage regression of complete hydranths and percentage organization of three-quarter developed hydranths to functionable animals. Conversely, when regression is decreased organization is decreased (Hammett and Elliott '35). Therefore—since regression was decreased by adenine organization also should have decreased—other things being equal. Not this but the opposite happened. Organization increased in the face of a diminished regression. This definite enhancement of organization is the outstanding developmental reaction to adenine. No other developmental phase is similarly affected in direction or extent and no other compound so far studied, be it amino acid or nucleic acid derivative, has shown this property. It follows that adenine or some part or derivative thereof is probably a specific participant in some process essential to organization expression.

These adenine results are of further importance. They support the principle of distinctiveness as applied to the part played by each of the naturally occurring amino acids and nucleic acid derivatives of general distribution in growth.

The purine nucleus on which the naturally occurring purine base adenine is built, is structurally compounded of pyrimidine and urea. Synthesis from such has actually been accomplished *in vitro* (Levene and Bass '31). The result is visualized in Plate 1, Fig. 1.

Now adenine is found in growing and other animal tissue(Kikkoji and Iguchi '07; Wells and Corper '10; Saiki '10; Wells and Long '13; Parnas '28). It is formed therein throughout development (Mendel and Saiki '08; Russo '27). It is also found in tissue of growing parts of plants (Totani '10; Kiesel '11; Miyake '24) and there is some evidence that plant growth utilizes pyrimidines of the barbituric acid series as purine precursors (Johnson '14).

The direct and inferential evidence indicates that the pyrimidine nucleus is an essential building block for adenine *in vitro* and *in vivo*.

It could therefore be expected that adenine might be endowed and express to some degree and in some direction some of the functional properties of the particular pyrimidine which contributes to its formation.

This should be cytosine for not only does this and no other naturally occurring pyrimidine have an amino group in position 6 as does adenine; but also the substituents and plan of positions 1, 2, and 3 in the enol form are closer to that of adenine than are those of uracil or thymine, Plate 1, Fig. 2. The significant configuration is enclosed in a dotted line.

Now cytosine inhibits expression of new growth initiation and forwards that of differentiation (Hammett, Lavine, and Lavine '37). Adenine also inhibits new growth initiation and it tends to forward differentiation. The implication is that these effects are due and specific to the cytosine moiety.

In the cytosine report the suggestion was made that inhibition of new growth initiation may reside in the introduction of the $NH_2$ group in position 6. This was because neither uracil nor thymine—which have no $NH_2$—showed this action. Yet both are pyrimidines. The fact that inhibition of new growth initiation carries over into adenine along with the carrying over of the $NH_2$ group in position 6 supports the idea.

The differentiation enhancing effect is attributable to the possibility of intermediary production of some pyrrolidone derivative as pointed out in the l-proline report (Hammett and Collings '37).

Purines are condensation products of pyrimidine and imidazole (Levene and Bass '31). Imidazole is a characterizing chemical group of the amino acid histidine, Plate 1, Fig. 3.

Because of this Cox and Rose ('26), Russo ('27), Calvery ('29), and others consider histidine a precursor of purines in development. The experimental evidence is reviewed and discussed by Mitchell and Hamilton ('29) and Cerecedo ('33).

Even though histidine be a precursor of adenine, it does not necessarily follow that the two are interchangeable. The report of Dubin and Corbitt ('25) that blood sugar is reduced by adenine and not by histidine, and the report of Cox and Rose ('26) that weight loss is not prevented when adenine is substituted for histidine in a diet deficient therein, are the to-be-expected results of such line of inquiry. Histidine has no pyrimidine nucleus; adenine is not an amino acid.

On the other hand it might be expected that with obelia adenine might express in some respect or other some functional property expressed by histidine, since both carry the imidazole group, and since loss of all specific physiological activity connected therewith may not necessarily follow condensation with cytosine.

The data show the expectation is realized for whereas cytosine is essentially non-toxic, its condensation product with imidazole is toxic and so is l-histidine (Hammett '35). It appears as if imidazole might be a source of adenine toxicity.

What is more important is that the outstanding property specific to l-histidine and found in no other amino acid or nucleic acid derivative so far studied

is also exhibited by adenine. This is the capacity for sustaining maintenance and holding back regression of the complete feeding hydranths.

This similarity in physiological activity would seem to be traceable to the similarity in chemical constitution and structure, the presence in each of imidazole.

And this suggests that the observed reaction is specific to imidazole, which permits the tentative allocation to this grouping of a definite physiological function—namely participation in the regulation of maintenance metabolism.

This finding confirms the l-histidine results and conclusions (Hammett '35) and extends them to definition of the responsible chemical grouping.

Thus the analysis contributed to the support of the premise that each and every naturally occurring tissue component of general distribution has its own individual and specific function in growth (Hammett '37); not only by confirming the previously reported results obtained with cytosine and l-histidine alone, but also by showing that these may be retained in their condensation product adenine in no insignificant degree.

What is more it allows presumptive allocation of definite function to definite chemical groupings, a thing which has hitherto neither been considered nor possible. This extension has been made possible largely from the protean nature of obelia growth expression. It is well to remark however that other factors may well intervene to mask or alter expression of these properties. In fact such should be expected since all processes have their balancing.

In 1913 Funk claimed adenine had a curative influence on beri-beri. This was denied by Voegtlin and White in 1916 and for irradiated adenine by Guha and Chakravorty in 1933. But Robbins *et al* in 1937 report curative effects on polyneuritis with pyrimidine supplemented with thiazole. Clearly, in the light of my data, these apparently conflicting results merit close examination with respect to the possibility that effective chemical groups may have been aborted by unstabilized variables. The general opinion that adenine cannot replace either vitamin $B_1$ or $B_2$ seems to be well founded however.

Although Noyon ('23) reports adenine is no potent factor in bacterial growth, Guha and Chakravorty ('33) found that the irradiated compound supported growth of 36 young rats which had stopped growing on a diet of starch—caseinogen—McCollum salt—cod-liver oil—and aqueous extract of ox kidney. Irradiated solenine, guanine, cytosine-Cl, and uracil had no such effect. Our data suggest this may be expression of the specific organization property, possibly supported by a sustaining of maintenance.

In the light of the postulate that SH is an essential participant in cell increase in number (Hammett '35a) it is of interest to correlate the fact that whereas uracil acts to favor proliferation of recurrent growths in obelia (Hammett '36) and to distort neutral sulfur excretion in the dog (Cerecedo '32), adenine which has no particular effect on proliferation expression in obelia, also has no significant effect on inorganic sulfur excretion (Cerecedo and Allen '34).

This is indirect support for the suggestion that uracil may be a specific

participant in some process which links together the preparatory chemical reactions for recurrence and those concerned in bringing this activity to fruition through proliferation.

Whatever the import of the correlation it emphasizes the fact of functional distinctiveness with respect to the nucleic acid derivatives.

Turning now to the possible relation of these findings to neoplasia it may or may not be significant that although both adenine and adenase may be present in tumors they also are noticeable for their sometime absence (Wells and Long '13: Long '14). Perhaps this may contribute to the defective organization which is a common characteristic of such pathological growths.

## SUMMARY AND CONCLUSION

From 69 experiments with close to 34,000 animals, half of which were exposed to adenine sulfate in concentrations ranging from M/200,000 to M/6250 adenine it was found that:—

1. Organization expression was enhanced.
2. Initiation expression was retarded.
3. Differentiation tended to be better than usual.
4. Proliferation tended to be less.
5. Maintenance was sustained and regression held back.
6. Catabolism was variable.

From these data it is possible to allocate participation in initiation retardation to that part of the pyrimidine configuration of cytosine which centers around the $NH_2$ group in position 6; to allocate participation in differentiation to some part or derivative of pyrrolidone; and to allocate participation in the sustaining of maintenance to imidazole. Expression of these properties may of course be masked or altered by other factors.

The conclusion is that adenine is a specific determinant of some chemical process essential to organization, and that super-imposed on this is a participation in growth regulation which derives from specificities inherent in the pyrimidine, cytosine, and imidazole components as separate factors.

These studies were made at the Marine Experimental Station of The Institute, North Truro, Massachusetts, with aid from Miss A. Bein of Philadelphia and the technical assistance of Misses Chatalbash, Elliott, Lavine, Steele, and Porter, and Messrs Lavine, Schlumberger, Reynolds, Collings, and Norris.

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# DEVELOPMENTAL GROWTH AND NUCLEIC ACID COMPONENTS VII. HYPOXANTHINE

*By*

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Levene and Bass ('31) state that hypoxanthine is an integral component of one of the nucleosides which enter into the composition of thymonucleic acid. Mathews ('31) on the other hand believes that hypoxanthine does not pre-exist in the nucleic acid molecule, but is derived from the adenine moiety thereof either by the action of acid *in vitro* or by the enzyme adenase *in vivo* before the nucleic acid is prepared. That adenine goes over to hypoxanthine with the greatest of ease *in vivo* is attested to by the reports of Kiesel ('11), Parnas ('28), Buell ('30), and others. If hypoxanthine is not a tissue component it is at least an immediate naturally occurring derivative of one and merits some attention with respect to its possible influence on growth.

TABLE 1

TREND OF TEST DEVIATION FROM CONTROL WITH RESPECT TO DEVELOPMENT AND METABOLIC ACTIVITY IN HYPOXANTHINE CULTURES. ALSO THE NUMBER OF COLONIES, HYDRANTHS, AND GONOPHORES AT THE STATED CONCENTRATIONS

| M/1000× | 200 | | 100 | | 50 | | 25 | | 12.5 | |
|---|---|---|---|---|---|---|---|---|---|---|
| No. Experiments | 10 | | 9 | | 15 | | 10 | | 10 | |
| | Cont. | Test | Cont. | Test | Cont. | Test | Cont. | Test | Cont. | Test |
| No. Colonies | 147 | 147 | 134 | 134 | 225 | 225 | 149 | 149 | 150 | 150 |
| No. Hydranths | 2403 | 2396 | 2230 | 2241 | 3669 | 3664 | 2468 | 2466 | 2554 | 2537 |
| No. Gonophores | 30 | 33 | 158 | 202 | 169 | 177 | 69 | 56 | 67 | 75 |

GROWTH

| | + | − | = | + | − | = | + | − | = | + | − | = | + | − | = |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Initiation | 4 | 1 | 2 | 2 | 2 | 3 | 5 | 2 | 0 | 2 | 1 | 4 | 0 | 2 | 3 |
| Proliferation | 4 | 3 | 1 | 1 | 4 | 3 | 2 | 3 | 3 | 4 | 3 | 1 | 5 | 1 | 2 |
| Differentiation | 2 | 5 | 3 | 3 | 4 | 3 | 4 | 3 | 2 | 1 | 5 | 4 | 4 | 3 | 2 |
| Organization | 3 | 3 | 3 | 1 | 5 | 2 | 3 | 4 | 2 | 3 | 1 | 4 | 3 | 1 | 5 |

METABOLISM (By Single Experiment)

| | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Maintenance | 4 | 5 | 1 | 4 | 3 | 2 | 5 | 8 | 2 | 2 | 7 | 1 | 3 | 7 | 0 |
| Catabolism | 4 | 3 | 3 | 4 | 3 | 2 | 10 | 4 | 1 | 3 | 6 | 1 | 1 | 7 | 2 |

METABOLISM (By Combined Experiments)

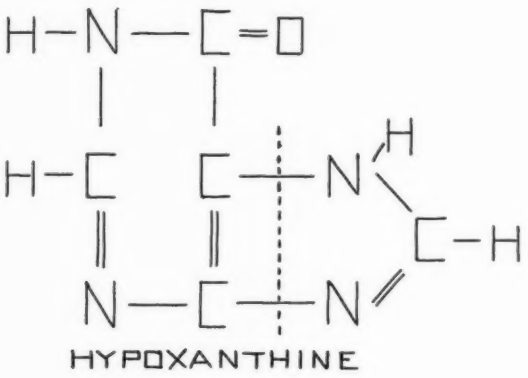
| | Cont. | Test | Cont. | Test | Cont. | Test | Cont. | Test | Cont. | Test |
|---|---|---|---|---|---|---|---|---|---|---|
| Regression | 45.1 | 45.6 | 45.1 | 44.5 | 48.9 | 53.4 | 36.3 | 39.8 | 33.4 | 37.2 |
| Catabolism | 79.0 | 79.2 | 82.3 | 84.4 | 69.1 | 71.6 | 80.6 | 77.9 | 82.6 | 79.7 |

Increase in Percentage Regression

| | | | | | |
|---|---|---|---|---|---|
| | 1.1 | −1.3 | 9.2 | 9.3 | 11.4 |

As usual the colonial-living form of the marine hydroid *Obelia geniculata* was the test animal. Details of procedure and description are referred to elsewhere (Hammett and Schlumberger '37). The hypoxanthine was prepared, identified, and purified by Schuchardt. Stock solutions containing 0.068 gram in 300 cc. fresh sea-water plus circa 0.6 cc N/10 NaOH for neutralization provided daily the aliquots from which test culture solutions were made. Fifty-four experiments were run with close to 26,000 animals, half of which were exposed to the compound in concentrations ranging from M/200,000 to M/12,500. Tests and controls were run simultaneously under like conditions of temperature, illumination, and pH. Four changes into fresh sea-water were made during the 24 hour course of each experiment. The data are given in Table 1. The method of its derivation, construction, and analysis is given in the report of Hammett and Schlumberger.

Fig. 1



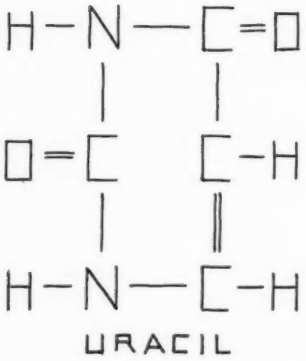




Fig. 2

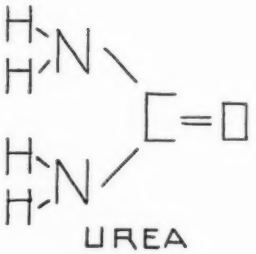



PLATE I

## RESULTS

There is no consistent evidence that hypoxanthine is toxic to obelia growth. The shift to retardation of catabolism at M/25,000 suggests a beginning toxicity (Hammett '36); but the evidence is too meagre to justify exclusion of these data. On the other hand since the trend increased at M/12,500 and was accompanied by increase in percentage of test regression it seems best to omit these data from the growth analysis. It is worth noting however that the trend of growth reaction to hypoxanthine is the same whether the data of M/25,000 and M/12,500 are included or excluded, singly or together. This is the to be expected result since no evidence is given that the compound is toxic to obelia growth in these experiments.

Maintenance was unaffected by hypoxanthine until M/50,000 when there was definite decrease in the percentage of test hydranths staying at the complete stage. The evidence is that test regression was less than control in 4 and greater in 3 of the 9 experiments done at M/100,000, while at M/50,000 it was greater in 8 and less in but 5 of the 15. Furthermore when all data are combined test regression at M/100,000 was 1.3% less than control, while at M/50,000 it was 9.2% greater. This direction and degree of difference persisted in the M/25,000 and M/12,500 levels.

New growth initiation was enhanced. Thus—bud formation in the tests was greater than control in 13 and less in but 6 of the 28 expressions given by the 4 sets of experiments. By concentrations it tended to be greater in 3 and less in none of the 4 series. And when the data are combined it was greater in 5 and less in but 1 of the 7 initiation potentialities. Closer analysis shows the enhancement was largely a matter of better recurrence. Thus—test recurrence was greater than control in 58 and less in but 17 per cent of the 12 expressions given by the 4 sets of experiments; while in the 16 other expressions of initiation test was greater than control in but 37 per cent and it was less in 25. Further, when the data are combined, test recurrence was greater than control in all 3 or 100 percent of its expressions, while of the 4 remaining expressions of initiation it was greater in 2, or but 50 per cent, and less in 1 and equal in 1.

Proliferation expression was essentially unaffected. Thus—bud change to half in the tests was greater than control in 11 and less in 13 of the 32 expressions given by the 4 sets of experiments. By concentrations it tended to be greater in 2 and less in 2 of the 4. And when the data are combined test was greater than control in 3, less in 2, and equal in 3 of the 8 proliferation potentialities.

Differentiation was retarded. Thus—half-stage change to three-quarter of the tests was less than control in 17 and greater in but 10 of the 40 expressions given by the 4 sets of experiments. By concentrations it tended to be less in 3 and greater in but 1 of the 4 series. And when the data are combined test was less than control in 5 and greater in but 3 of the 10 differentiation potentialities. This was essentially a hydranth reaction. Gonophore differentiation was better than usual. Thus—in the 8 expressions given by the 4 sets of experiments test was greater than control in 5 and less in but 2. By concentrations it was greater

in 3 and less in but 1 of the 4 series. And when the data are combined test was greater in both of the 2 potentialities.

Organization expression was essentially unaffected. Thus—test change from the three-quarter stage to the complete functionable hydranth was greater than control in 10 and less in 13 of the 36 expressions given by the 4 sets of experiments. By concentrations it tended to be greater in 1, less in 1, and equal in 2 of the 4 series. And when the data are combined test was greater than control in 3 and less in 4 of the 9 organization potentialities.

There is some indication that regeneration was better in the hypoxanthine than control cultures. Thus—new bud formation from broken pedicels in the tests was greater than control in 3 of the 4 sets of experiments. Further it was greater in 25 and less in but 17 of the 44 experiments.

## DISCUSSION

Just as adenine was taken to be a condensation product of the pyrimidine cytosine and imidazole; so may hypoxanthine be taken as a condensation product of imidazole and the pyrimidine uracil. For hypoxanthine may be considered as adenine in which the $NH_2$ of position 6 has been replaced with an oxygen with an added hydrogen in position 1; and uracil may be considered as cytosine in which the $NH_2$ of position 6 has been replaced with an oxygen with an added hydrogen in position 1, Chart 1, Fig. 1.

Uracil rather than thymine is taken as the pyrimidine baustein of hypoxanthine because of the presence in the latter of a $CH_3$ group instead of hydrogen in position 7 (Levene and Bass '31). It might therefore be expected—other things being equal—that the purine hypoxanthine would exhibit some of the properties of its constituent pyrimidine with respect to its influence if any on obelia growth (Hammett '38).

This expectation is realized for the growth reaction to hypoxanthine simulates in significant respects that of uracil. Recurrence is specifically enhanced and differentiation retarded by hypoxanthine as by uracil; while in neither case is either proliferation or organization seriously affected (Hammett '36).

The correspondence is emphasized by the fact that these compounds and no others show this particular combination of growth influence. It is evident that these reactions to hypoxanthine are determined by its uracil moiety.

This not only confirms the uracil results as such; it also gives evidence similar to that adduced by the adenine data that the pyrimidine constituent sets the pattern for the growth response to purine.

Now hypoxanthine also carries the imidazole ring and there is evidence that this may act to support maintenance and retard regression. At least such reaction occurred in the presence of l-histidine (Hammett '35) and adenine (Hammett '38); two compounds whose similarity lies only in the common presence of imidazole. But: as these data show, hypoxanthine has no sustaining action on maintenance; indeed it tends in higher concentration to forward regression.

This absence of effect does not necessarily negative the positive associations shown by the other compounds. These stand as mutually sustaining evidence of

specificity of influence attributable to imidazole as a definite chemical grouping.

More than one factor may have masked or prevented the effect. Thus there was definite suggestion that maintenance tended to be consistently less in the presence of uracil (Hammett '36). So here it may be the influence of this pyrimidine which is masking the imidazole effect.

Then it is probable that hypoxanthine is a first step in the oxidation of adenine (Kiesel '11; Wells and Long '13; Parnas '28). It is possible that the change in state of oxidation of position 6 contiguous to the imidazole ring may have neutralized its maintenance sustaining effect by increasing relative acidity (Benedict '12).

Or once the oxidation has begun it may be that it rapidly sweeps on through xanthine, uric acid, and allantoin to urea (Mathews '31), and thus expression of the imidazole effect is prevented.

Now it is known that under certain rigorous treatment hypoxanthine will yield glycine (Levene and Bass '31). Glycine has been shown to stimulate regeneration. Regeneration is stimulated in these hypoxanthine experiments. Glycine also has a high regression activity. And regression tends to increase in hypoxanthine cultures. It is not impossible that obelia can easily do *in vivo* what the chemist finds hard to do *in vitro*—and under certain conditions produce glycine from hypoxanthine. If this should happen—and the fact that regeneration is stimulated suggests that glycine has been produced because this and no other amino acid or nucleic acid derivative has had this effect—it is clear that the great regression activity of glycine would be more than sufficient to overcome the mild sustaining influence of imidazole thereon and to shift a retardation of regression to enhancement.

Finally it is possible hypoxanthine is broken down in intermediary metabolism along a different line of cleavage than is adenine.

Note was made in an earlier report that uracil lacks the property of differentiation enhancement possessed by the other two naturally occurring pyrimidines (Hammett and Collings '37). The purine data bring confirmation of this difference for differentiation was retarded in the presence of the uracil-carrying hypoxanthine, and forwarded in the presence of the cytosine-carrying adenine.

The establishment of this difference as a fact dispels any notion the reaction in the uracil experiments was due to contamination. It puts the difference squarely on uracil as such. It validates the suggestion that uracil gives by-products in intermediary metabolism which differ from those given by cytosine and thymine. Uracil apparently does not give rise to pyrrolidone, or some part or derivative thereof which seemingly contributes to differentiation (Hammett '37).

This suggests that uracil is the factor which determines the difference in breakdown of hypoxanthine in intermediary metabolism. The cumulative evidence indicates that uracil is the primary determinant of hypoxanthine influence on growth.

From all this the thesis develops that hypoxanthine splits into uracil and

urea, Chart 1, Fig. 2. Thus the imidazole ring is put hors de combat. This is the most logical present interpretation of the fact that maintenance is not sustained and the growth reaction is essentially that of uracil.

Indeed insofar as developmental activity is concerned the growth influence of hypoxanthine is quite the same as that of uracil. This might not be unexpected since as the preponderance of evidence would seem to indicate, the compound is actually not an integral component of living tissue but simply a close derivative thereof.

Additional evidence is had from the fact that with loss of the $NH_2$ group in position 6 there is concomitant loss of retarding effect on new growth initiation. This is consistent with the postulate that the group association is a significant factor in this particular developmental growth activity (Hammett, Lavine, and Lavine '37; Hammett '38).

Some minor specificities however do serve to distinguish the two compounds and bestow individuality on hypoxanthine. This in the sense that no other compound shows a like combination of growth influence. Thus—in addition to the uracil effect of enhancing recurrence and retarding differentiation—hypoxanthine seems to act as a stimulus to regeneration, and quite definitely steps up that change in gonophore development which has been attributed to differentiation. This may be evidence of a specificity for a type of growth peculiar to medusa production. It is possibly associated with the purine configuration since it is also shown with adenine.

Finally there is a hint of specificity in catabolic activity. This tends to be slightly enhanced before it begins to give evidence of toxicity. Thus disintegration of senile animals in the tests is greater in 18 and less in but 10 of the 29 experiments done in the M/200,000–M/50,000 range. By concentrations it tends to be greater in all three series. If this is a true trend it is consistent with the finding of Underhill and Farrell ('22) that hypoxanthine injected into fasting rabbits led to an augmented excretion of total nitrogen, creatine, and creatinine; evidence of increased protein catabolism.

The occurrence of hypoxanthine in tissues is, as would be expected, essentially the same as that noted for adenine of which it is the naturally occurring derivative (Hammett '38). Although it occurs during development (Mendel and Saiki '08) it has shown no potency in bacterial growth (Noyon '23) and as the hydrochloride it has been reported to act inhibitively on both growth and cell division in plants (Coppin '13). These results are not to be taken too seriously however since the concentrations used were probably toxic.

It is worth noting that hypoxanthine has been found in octopus blood and urine (Hoppe-Seyler and Linneweh '31) and that it is present in tumor tissue only in very small amounts (Saiki '10; Long '14).

## SUMMARY AND CONCLUSION

From 54 experiments with close to 26,000 animals, half of which were exposed to hypoxanthine in concentrations ranging from M/200,000 to M/12,500 it was found that:—

1. Recurrent growth expression was enhanced.
2. Differentiation expression was retarded.
3. Proliferation and organization were essentially unaffected.
4. Catabolism may have been slightly enhanced.
5. Maintenance tended to be cut and regression increased in higher concentrations.
6. There was no toxic action on growth.

The conclusion is that the participation of hypoxanthine in developmental activity is determined by its pyrimidine constituent uracil.

The implication of this with respect to cleavage of the molecule in intermediary metabolism is discussed in the text.

Hypoxanthine, however, like all other compounds, does have its own influence. Its individuality resides in its effect on single growth expressions rather than on any basic developmental activity. For—in addition to its uracil effect it seems to forward regeneration and gonophore differentiation.

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# DEVELOPMENTAL GROWTH AND NUCLEIC ACID COMPONENTS VIII. XANTHINE

*By*

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There is no sure evidence that xanthine is an integral component of nucleic acid. It is however an immediate derivative both *in vitro* and *in vivo* of the tissue baustein guanine (Levene and Bass '31; Kiesel '10; Mathews '31).

Evaluation of its influence on growth was primarily undertaken in order that comparison could be had between this and that of the lesser oxidized hypoxanthine, and to see if these immediate derivatives of the naturally occurring tissue components retained any of the growth affecting properties of their antecedents.

As will be seen, data have been obtained which are of interest not only in these directions, but also in connection with the manner of cleavage of these compounds in hydroid intermediary metabolism (Hammett and Steele '38).

As usual the colonial-living form of the marine hydroid *Obelia geniculata* was the test animal. Details of description and procedure are referred to elsewhere (Hammett and Schlumberger '37). The xanthine was prepared, identified, and purified by Schuchardt. Stock solutions containing 0.076 gram in 500 cc fresh well-water provided daily the aliquots from which test culture solutions were made. Equivalent amounts of freshly drawn well-water were added to the test and control sea-water cultures. Fifty experiments were run with close to 24,000 animals, half of which were exposed to the compound in concentrations ranging from M/200,000 to M/12,500. Tests and controls were run simultaneously under like conditions of temperature, illumination, pH, and salinity. Four changes into fresh sea-water were made during the 24 hour course of each experiment. The data are give in Table 1. The method of its derivation, construction, and analysis is given in the report by Hammett and Schlumberger ('37).

## RESULTS

Xanthine is pretty definitely toxic to obelia growth at M/12,500. The evidence is that of the 34 potentialities for growth expression, test was less than control in 13 and greater in but 7; while in the preceding lower M/25,000 concentration test was less than control in 11, greater in 11, and equal in 10. Analysis of the growth reaction will therefore be based on the data of M/200,000 to M/25,000 inclusive.

New growth initiation was enhanced. Thus—bud formation in the tests was greater than control in 9 and less in but 5 of the 28 expressions given by the 4 sets of experiments. By concentrations it tended to be greater in 3 and less in

but one of the 4 series. And when the data are combined it was greater in 4 and less in 3 of the 7 potentialities for activity expression.

Closer analysis however shows the enhancement was a matter of better recurrence. Thus—test recurrence was greater than control in 7 and less in but 1 of the 12 expressions given by the 4 sets of experiments; while in the 16 other expressions of initiation test was less than control in 4 and greater in but 2. Further, when the data are combined, test recurrence was greater than control in all 3 or 100 per cent of its expressions, while of the 4 remaining expressions of initiation test was less than control in 3 and greater in but 1. This differential persists into the M/12,500 concentration.

TABLE 1

TREND OF TEST DEVIATION FROM CONTROL WITH RESPECT TO DEVELOPMENTAL AND METABOLIC ACTIVITY IN XANTHINE CULTURES. ALSO THE NUMBER OF COLONIES, HYDRANTHS, AND GONOPHORES AT THE STATED CONCENTRATIONS

| M/1000× | 200 | | 100 | | 50 | | 25 | | 12.5 | |
|---|---|---|---|---|---|---|---|---|---|---|
| No. Experiments | 10 | | 10 | | 10 | | 10 | | 10 | |
| | Cont. | Test | Cont. | Test | Cont. | Test | Cont. | Test | Cont. | Test |
| No. Colonies | 150 | 150 | 147 | 147 | 147 | 147 | 150 | 150 | 150 | 150 |
| No. Hydranths | 2434 | 2446 | 2405 | 2409 | 2510 | 2496 | 2457 | 2452 | 2495 | 2492 |
| No. Gonophores | 133 | 140 | 170 | 115 | 87 | 77 | 124 | 89 | 97 | 87 |
| **GROWTH** | | | | | | | | | | |
| | + − = | + − = | + − = | + − = | + − = | + − = | + − = | + − = | + − = | + − = |
| Initiation | 0 1 6 | 4 2 1 | 3 1 3 | 3 2 3 | 2 1 4 | 3 2 2 | | | | |
| Proliferation | 4 1 3 | 3 3 2 | 3 2 3 | 2 3 3 | 3 3 2 | 2 3 3 | | | | |
| Differentiation | 2 4 4 | 5 3 2 | 2 4 4 | 3 5 1 | 2 5 3 | | | | | |
| Organization | 1 5 3 | 0 2 7 | 2 3 4 | 3 2 4 | 0 3 5 | | | | | |
| **METABOLISM (By Single Experiment)** | | | | | | | | | | |
| Maintenance | 3 5 2 | 5 5 0 | 3 5 2 | 1 7 2 | 7 2 1 | | | | | |
| Catabolism | 7 3 0 | 7 3 0 | 2 5 3 | 2 6 2 | 5 3 2 | | | | | |
| **METABOLISM (By Combined Experiments)** | | | | | | | | | | |
| | Cont. | Test | Cont. | Test | Cont. | Test | Cont. | Test | Cont. | Test |
| Regression | 34.3 | 36.1 | 44.1 | 43.5 | 42.3 | 45.2 | 30.0 | 31.5 | 28.4 | 25.2 |
| Catabolism | 82.2 | 84.2 | 74.8 | 79.1 | 85.7 | 84.3 | 88.2 | 88.8 | 77.7 | 80.6 |
| **Increase in Percentage Regression** | | | | | | | | | | |
| | 5.0 | | −1.6 | | 6.9 | | 4.6 | | −11.2 | |

Proliferation expression tended towards enhancement. Thus—bud change to half in the tests was greater than control in 13 and less in but 9 of the 32 expressions given by the 4 sets of experiments. By concentrations it tended to be greater in 2 and less in none of the 4 series. And when the data are combined it was greater in 4 and less in 2 of the 8 potentialities. This too tended to cluster around recurrence. For here test proliferation was less than control in but 3 and greater in 6 or 50 per cent of the 12 expressions given by the 4 sets of experi-

ments, while of the remaining 20 it was less in all of 6 and greater in but 5 or 25 per cent.

Differentiation was retarded. Thus—half stage change to three-quarter of the tests was less than control in 16 and greater in but 12 of the 40 expressions afforded by the 4 sets of experiments. By concentrations it was less in 3 and greater in but 1 of the 4 series. And when the data are combined it was greater in but 1 and less in 3 of the 10 potentialities. This was essentially a hydranth reaction. It was not exhibited by the gonophores.

Organization was retarded. Thus—three-quarter change to complete functionable hydranths in the tests was less than control in 12 and greater in but 6 of the 36 expressions given by the 4 sets of experiments. By concentrations it tended to be less in 3 and greater in but 1 of the 4 series. And when the data are combined it was less in 3 and greater in but 1 of the 9 potentialities for activity expression.

Regeneration tended to be better in the presence of xanthine. Thus—new bud formation on broken pedicels was greater in 2 and less in none of the 4 sets of experiments. Further test regeneration was greater in 17 and less in but 14 of the 40 experiments.

In general maintenance was uncertain; while catabolism tended to be greater in the lower and less in the higher concentrations.

## DISCUSSION

Xanthine can lay no claim to great individuality with respect to growth influence. The retardation of organization expression in its presence seems to be its sole distinguishing mark among the nucleic acid derivatives. All other effects are traceable to other configurations.

This does not mean the picture is unilluminating. Indeed quite the contrary. The data when correlated with other findings not only suggest that the purines hypoxanthine and xanthine undergo cleavage in obelia intermediary metabolism along a line of fracture which differs from that of adenine; but also suggest a particular chemical group as being concerned in a particular developmental activity; viz., new growth initiation by recurrence.

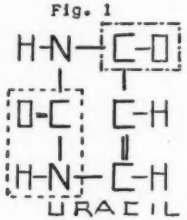
It is possible that the trend to specific enhancement of gonophore differentiation expression exhibited in both the adenine and hypoxanthine experiments is essentially a purine effect (Hammett and Steele '38). The fact that gonophore differentiation did not follow hydranth differentiation to retardation in the presence of xanthine might be taken to indicate the persistence of this effect in the presence of this compound also. It is the only growth reaction attributable to purine since it is the only one common to all three.

Since the reaction is that of a single structure instead of all those capable of going on to differentiation, it is peculiar to the structure and not to differentiation as such (Hammett '36). Thus it cannot be said that purine is specially concerned in differentiation. What can be said is that purine is possibly of special importance to some process essential to some particular phase of medusa development. Verification must await further inquiry.

Xanthine, like hypoxanthine, acts to enhance regeneration. This may be attributed to a possible glycine production (Hammett and Steele '38). The implication will be shown presently.

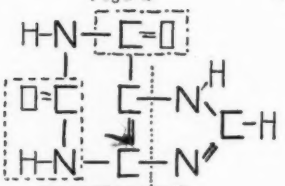
So far we have been dealing with minor specificities. Turning to the general developmental growth reaction it is seen that xanthine produced essentially

Fig. 1



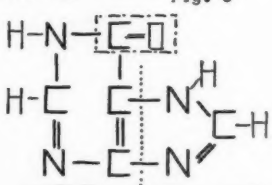
URACIL

Fig. 2



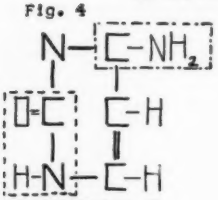
XANTHINE

Fig. 3



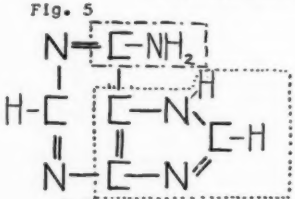
HYPOXANTHINE

Fig. 4



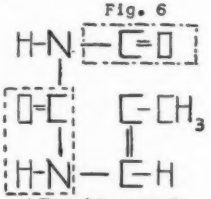
CYTOSINE

Fig. 5



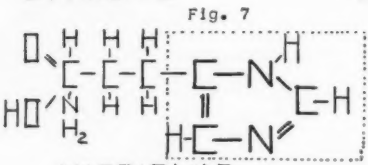
ADENINE

Fig. 6



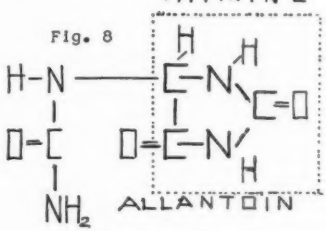
THYMINE

Fig. 7



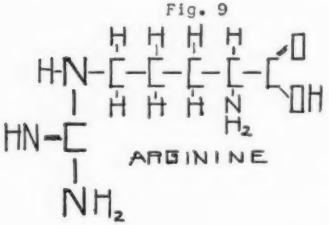
HISTIDINE

Fig. 8



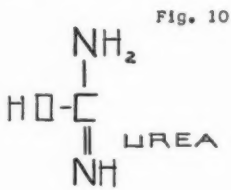
ALLANTOIN

Fig. 9



ARGININE

Fig. 10



UREA

PLATE I

the same effect as uracil. In its presence, as in that of uracil, initiation by recurrence is favored; proliferation of recurrent growths is favored; and differentiation is retarded.

Hypoxanthine also simulated uracil in its effect (Hammett and Steele '38). But xanthine appears to come somewhat closer in this than hypoxanthine. It acts to forward proliferation of recurrent growths; while hypoxanthine has no such effect (Hammett '36a).

If the reader will look at the accepted formula for hypoxanthine in Fig. 3 Plate 1, and compare it with that for uracil in Fig. 1, he will see that although the pyrimidine component is close kin to uracil it still is no exact replica thereof. It has no oxygen in position 2 and no hydrogen in position 3. If now the reader will compare xanthine of Fig. 2 with uracil he will see that here correspondence in all free positions is perfect, viz., in positions 1, 2, 3, and 6.

That this difference should be reflected in the respective growth influences of the two purines is to say the least most gratifying. That the uracil-purine should act in growth as does uracil; while the almost-uracil-purine acts almost but not quite as uracil is solid foundation for the essential verity of these results.

To recapitulate—xanthine of perfect uracil contour produces like uracil a forwarding of new growth initiation by recurrence, a forwarding of proliferation in recurrent growths, and a retardation of differentiation; but hypoxanthine of imperfect uracil contour, though it produces like uracil a forwarding of new growth initiation by recurrence, and a retardation of differentiation, *fails to forward recurrent growth proliferation.*

Therefore since present concepts state that xanthine differs from hypoxanthine only in having carbonyl instead of methine in position 2, and imid instead of naked nitrogen in position 3, it would seem as if it is this association which is responsible for the proliferation effect of xanthine.

Consistent is the fact that not only uracil but also cytosine and thymine possess this arrangement and they also act to forward proliferation of recurrent growths (Hammett, Lavine and Lavine '37; Hammett and Lavine '37), while adenine which does not possess the group has no such effect (Hammett '38). Significant is the fact that arginine—potentially a source of uracil—also acts to forward recurrent growth proliferation (Hammett and Chatalbash '35).

Taking the data as a whole it is probably no great deviation from fact to allocate to the $\overset{2}{\underset{O}{-C-}}\overset{3}{\underset{H}{-N-}}$ pyrimidine conjunction a specific participation in some process concerned in proliferation of recurrent growth. That other properties may be exhibited—alone or in combination—is not excluded by this definition. That contiguous groupings may modify its expression is to be understood.

Xanthine, hypoxanthine, and uracil have the common faculty of enhancing initiation of recurrent growths. This also can be traced to a common denominator as was done for the proliferation factor.

The cytosine and adenine data suggest that position 6 in the pyrimidine

nucleus is important in this connection. The postulate comes from the fact that introduction of $NH_2$ in this position is accompanied by marked inhibition of initiation (Hammett, Lavine and Lavine '37; Hammett and Lavine '37), a reaction which *does not* obtain in the presence of xanthine, hypoxanthine, uracil, and thymine (Hammett and Steele '38; Hammett '36a; Hammett and Lavine '37), in none of which is there an $NH_2$ in position 6 (Levene and Bass '31), Plate 1, Figs. 1, 2, 3, 4 and 6.

When however position 6 carries oxygen instead of $NH_2$ as it does in the case of xanthine, hypoxanthine, and uracil, there is not only *no retardation* of recurrent growth initiation; but instead there is enhancement. Since this is general, with the exception of thymine—which is a special case probably because of the damping effect of $CH_3$ in position 5—it would seem to justify the conclusion that around position 6 there clusters an association of peculiar significance to new growth initiation.

The enhancement property of these compounds seems to condense around recurrent growth. Though position 6 is undoubtedly a center of influence for initiation in general, it looks as if oxygen thereon weights the influence towards a specific group of initiatory developmental processes, rather than towards all; i.e., towards recurrence more than towards new growth initiation from anlagen; broken pedicels by regeneration; or cut ends of hydrocaulae by vasculogenesis.

Something else is probably needed to broaden the influence to embrace all expressions of initiation. Perhaps it depends upon the equilibrium state of the keto-enol tautomerism. Perhaps it is determined by whether the nitrogen in position 1 is naked or carries a hydrogen. These questions need further study. What can be said at present, and this is no small step forward, is that position 6 in the pyrimidine nucleus carries a factor of specific importance to initiation; that an oxygenated position 6 is a determining participant in some process essential to enhancement of recurrent growth initiation; and that the $\overset{1}{\underset{H}{-N-}}\overset{6}{\underset{O}{-C-}}$ combination is probably a modifying influence.

* * *

From this and what has gone before it is evident that the pyrimidine moiety is a dominant factor in the growth influence of xanthine. Since the same held true for both hypoxanthine and adenine it begins to look as if this is true for the naturally occurring purines in general. Consistent is the fact that xanthine did not act to support maintenance. The absence of this effect may be interpreted as was the like phenomenon in the hypoxanthine study (Hammett and Steele '38). There is however a hint that this may not have been far away. Regression was surely less by some 11 per cent in the M/12,500 concentration. If this was real it might have come from a lessened tendency to the assumed production of glycine. The evidence is that whereas test regeneration from broken pedicels in the hypoxanthine study was better than control in 75 per cent of the 4 sets of experiments; in the xanthine study it was better in but 50 per cent. And

whereas it was better in 57 percent of the 44 experiments with hypoxanthine, in the 40 with xanthine it was better in but 42 percent. All this is merely suggestive.

It is to be noted that although there is the to be expected difference between the growth effect of adenine and that of hypoxanthine and xanthine because of the difference in the pyrimidine components; there is on the other hand no concordance as might be expected with respect to the alleged influence of the imidazole group of common possession. To this grouping may be attributed the property of acting to sustain maintenance. It and the reaction thereto are characterizingly common to l-histidine, allantoin, and adenine (Hammett '35; Hammett '38; Hammett and Elliott '36). Of the three purines only adenine acts to sustain maintenance, Plate 1, Figs. 5, 7 and 8.

The purines hypoxanthine and xanthine therefore differ from the purine adenine with respect to a basic property associated with a definite chemical group of common possession.

The difference merits examination because in it may lie a matter of fundamental importance to differential intermediary metabolism. In this connection it may be noted that xanthine acts to produce a greater drop in blood sugar of rabbits than does adenine (Durbin and Corbitt '25).

There are two chief possibilities. Either the maintenance supporting effect of imidazole is blanketed by the opposing effect of some nearby group; or the molecular cleavage of intermediary metabolism produces different by-products because it is along a different line of fracture.

The first assumes the observed effects are produced by intact molecules. The evidence is speculative.

Granting the possibility, however, it is seen from the formulae that the sustaining influence occurs when oxygen is absent from purine position 6, or in the case of histidine and allantoin from the side-chain position immediately adjacent to the imidazole group, Plate 1, Figs. 5, 7, and 8. Yet allantoin with imidazole carrying oxygen on two carbons still acts to support maintenance (Hammett and Elliott '36). This detracts somewhat from the idea that carbonyl oxygen is a blanketing influence.

It may be that nearby amino or imid groups without or even with interposition of carbonyl are positive factors for expression. Such are common to adenine, histidine, and allantoin. Consistent with the inference is the suggestion that cytosine tends to support maintenance (Hammett, Lavine and Lavine '37). But this may be nothing more than a result of synthesis into adenine.

The question is unanswerable at present. Only the suggestion can be made that if the absence of maintenance support is derived from blanketing of imidazole by nearby groups, it lies in all probability in the carbonyl of position 6, or the absence of supporting amino or imid groupings.

The idea that hypoxanthine and xanthine undergo a different type of cleavage than adenine in obelia intermediary metabolism has support.

It would be strange indeed if the nuclear components of obelia differed from those of other organisms and existed as static and fixed inactive compounds.

The fact that these studies show them to be potent influences for growth indicates they are far from being immobile tissue constituents.

It is well known that enzymes exist which split nucleic acid; which oxidize the products such as adenine and guanine to hypoxanthine and xanthine; the hypoxanthine to xanthine; the xanthine to uric acid; the uric acid to allantoin; and with hydrolysis the allantoin to urea, Plate 1, Figs. 5, 3, 2, 8 and 10, (Mathews '31).

Purines may be considered not only as condensation products of urea with pyrimidine, but also of imidazole with pyrimidine precursors (Levene and Bass '31). Synthesis *in vitro* follows the first course, enzyme de-synthesis *in vivo* may follow the second (Goldschmidt '14).

It is evident that there inheres in purine the potentiality for direct splitting into urea and pyrimidine, and for oxidation with freeing of imidazole and urea via allantoin, Plate 1, Figs. 8 and 10. The first possibility is shown by the dotted line in the formulae for xanthine and hypoxanthine. The second in that for adenine. The freeing of the imidazole nucleus in allantoin is indicated by the dotted line in Fig. 8.

Both potentialities seem to be expressed in the intermediary metabolism of these purines by obelia. Thus the maintenance support by adenine can be interpreted as resulting from allantoin formed in the classical oxidation sequence; and the absence of maintenance support by hypoxanthine and xanthine can be interpreted as indicating that in obelia these compounds do not follow the classical formula of oxidation and hydrolysis to allantoin, but instead split along a different line of fracture to form uracil and urea.

Support for this may be found in the reports of Wells '12; Long '14; Wells and Corper '10; Wells and Long '13, that all tissues do not have the power to change xanthine to uric acid; in higher animals only the liver seems to possess this property.

But again decision is unsure. For the question may be asked as to why no xanthine effect is exhibited in the adenine results if adenine passes through xanthine on its way to allantoin; and the question may be raised as to the source of the glycine effect if hypoxanthine and xanthine do not go to allantoin, since presumably glycine is a natural by-product of allantoin hydrolysis through allanturic acid and hydantoin (Mathews '31). What can be said however is that despite suggestion the intermediary metabolism of hypoxanthine and xanthine by obelia may differ from that of adenine; the possibility can not be evaded the observed differences in obelia growth and metabolic activity are due to compositional differences *per se*.

* * *

Xanthine is found in plant and animal tissues only in traces and to much less extent than the other purines (Kiesel '10; Wells and Corper '10; Saiki '10; Long '14). According to Weevers ('30) the derivatives caffeine and theobromine are most abundant in the growing parts of plants and decrease with age.

Though Coppin ('13) reports xanthine acts to inhibit growth and cell division in plants, Ciamician and Ravenna ('18) got no evidence of toxicity to

seedlings, and Schreiner and Reed ('08) found actual benefit in low concentrations up to 22 ppm. Further Frei and Riedmüller ('31) observed slight stimulation. These findings are in agreement with ours which show stimulation at the proper concentration and toxic effects at higher.

## SUMMARY AND CONCLUSION

From 50 experiments with close to 24,000 animals (Obelia geniculata) half of which were exposed to xanthine in concentrations ranging from M/200,000 to M/12,500 it was found that:—

1. Recurrent growth initiation was enhanced.
2. Recurrent growth proliferation was enhanced.
3. Differentiation and organization expression were retarded.
4. Catabolism and maintenance were essentially unaffected.
5. There was toxic action on growth at M/12,500.

The conclusion is that the participation of xanthine in developmental activity is determined by its pyrimidine component uracil.

The individuality lies in the combination of the uracil effect with a retarding effect on organization, and a trend to the production of enhanced regeneration.

The implications are discussed in the text.

The distinction from hypoxanthine lies in the favoring influence on recurrent growth proliferation.

The data allow allocation of a conditioning influence on new growth initiation to position 6 of the purine molecule; of a retarding influence when $NH_2$ is carried thereby; and a forwarding influence on that of recurrent growth when oxygen is the substituent. They also allow allocation to the $-\overset{2}{\underset{O}{C}}—\overset{3}{\underset{H}{N}}-$ combination of a determining participation in proliferation of recurrent growths.

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# A CASE OF BIOLOGICAL PERIODICITY

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## INTRODUCTION

Examples of periodicities in biology have been known for a long time; in fact, some of the most obvious and fundamental biological phenomena (such as reproduction, the heart beat, etc.) are periodic in character. Inasmuch as periodical changes in the cell are found to be connected with the rhythm of cellular division, a study of such periodicities is of particular interest for a general theory of cellular multiplication and growth. N. Rashevsky has shown that on the basis of a certain postulated mechanism, the possibility of periodic variation of concentration of metabolizing substances in a cell could be demonstrated. However, in his discussion Rashevsky has confined himself to the case in which the diffusion coefficients inside and outside the cell are infinite.

In this paper we propose to examine the more general case in which the external diffusion coefficient is infinite while the internal coefficient is finite. We shall consider, with Rashevsky, a case in which two substances are being metabolized in a spherical cell. We shall suppose the reactions involving the two substances to be so linked that the rate of production of one substance per unit volume is proportional to a linear combination of the concentrations of both substances. Thus if $c_1$ and $c_2$ are the concentrations of the two substances, then

$$\text{rate of production of } c_1 = A_{11}c_1 + A_{12}c_2$$
$$\text{rate of production of } c_2 = A_{21}c_1 + A_{22}c_2.$$

Depending on the signs of the coefficients, several cases can be distinguished. For example, if all the $A$'s are negative, the two substances are being consumed; if $A_{11}$, $A_{22}$ are negative and $A_{12}$, $A_{21}$ are positive, the rate of production of one substance is proportional to its own concentration while its rate of consumption is proportional to the concentration of the other.

The setting up of the partial differential equations which govern the variations in concentration follows immediately from previous work. Assuming the internal coefficients of diffusion constant, we have for the amount of each material flowing into a small volume element $\Delta x \Delta y \Delta z$

$$D_i \nabla^2 c_i \Delta x \Delta y \Delta z.$$

If the change in concentration per second per unit volume is $\partial c_i/\partial t$, then to have a material balance inside the cell

$$\left.\begin{aligned} \frac{\partial c_1}{\partial t} &= D_1 \nabla^2 c_1 + A_{11}c_1 + A_{12}c_2 \\ \frac{\partial c_2}{\partial t} &= D_2 \nabla^2 c_2 + A_{21}c_1 + A_{22}c_2 \end{aligned}\right\}. \tag{1}$$

In the general case of finite external $D$ a similar set of equations with all the $A$'s $=0$ would hold outside the cell. However, we shall assume infinite external $D$'s for simplicity; this implies that the concentrations are constant $(=c_{0i})$ everywhere outside the cell. Such an assumption corresponds physically to turbulence in the medium surrounding the cell.

If we consider our cell to be spherical with center at the origin, the $c_i$ become functions of $r$ alone and equations (1) become

$$\left.\begin{aligned} \dot{c}_1 &= D_1 c_1'' + \frac{2}{r} D_1 c_1' + A_{11} c_1 + A_{12} c_2 \\ \dot{c}_2 &= D_2 c_2'' + \frac{2}{r} D_2 c_2' + A_{21} c_1 + A_{22} c_2 \end{aligned}\right\} \tag{2}$$

where dots represent time derivatives and primes derivatives with respect to $r$. As boundary conditions we take

1. At center of cell, concentrations remain finite:

$$\text{i.e.,} \qquad c_1\Big|_{r=0}, \qquad c_2\Big|_{r=0} \qquad \text{finite.}$$

2. At surface of cell $r=r_0$

$$D_1 c_1'\Big|_{r_0} = h_1(c_{01} - c_1)\Big|_{r_0}$$

$$D_2 c_2'\Big|_{r_0} = h_2(c_{02} - c_2)\Big|_{r_0}$$

where $h_1$ and $h_2$ are the permeabilities of the cell membrane for the two substances. Equations (2) can be simplified by the usual substitution $u_i = c_i r$ to

$$\left.\begin{aligned} D_1 u_1'' &= \dot{u}_1 - A_{11} u_1 - A_{12} u_2 \\ D_2 u_2'' &= \dot{u}_2 - A_{21} u_1 - A_{22} u_2 \end{aligned}\right\} \tag{3}$$

with boundary conditions

$$\left.\begin{aligned} &1.) \quad u_1(0) = u_2(0) = 0 \\ &2.) \quad u_1'\Big|_{r_0} = \frac{D_1 - h_1 r_0}{D_1 r_0} u_1\Big|_{r_0} + \frac{h_1 r_0 c_{01}}{D_1} \\ &\qquad u_2'\Big|_{r_0} = \frac{D_2 - h_2 r_0}{D_2 r_0} u_2\Big|_{r_0} + \frac{h_2 r_0 c_{02}}{D_2} \end{aligned}\right\}. \tag{4}$$

*Solution of Equations*

A. Time independent equation.

The solution of (3) will in general consist of a linear combination of a function

involving the time and a function independent of time. Thus we may assume

$$u_i = w_i(r, t) + y_i(r) \tag{5}$$

where $y_i$ are the solutions of

$$\left.\begin{aligned} D_1 y_1'' &= -A_{11}y_1 - A_{12}y_2 \\ D_2 y_2'' &= -A_{21}y_1 - A_{22}y_2 \end{aligned}\right\} \tag{6}$$

with suitable boundary conditions, and $w_i$ are the solutions of (3) with $u$ replaced by $w$. The boundary conditions are easily found by putting (5) into (4) and equating time dependent and independent parts. Thus

$$\left.\begin{aligned} &1.)\quad y_1\Big|_{r=0} = y_2\Big|_{r=0} = 0 \\ &2.)\quad y_1'\Big|_{r_0} = \frac{D_1 - h_1 r_0}{D_1 r_0}\, y_1\Big|_{r_0} + \frac{h_1 r_0 c_{01}}{D_1} \\ &\qquad y_2'\Big|_{r_0} = \frac{D_2 - h_2 r_0}{D_2 r_0}\, y_2\Big|_{r_0} + \frac{h_2 r_0 c_{02}}{D_2} \end{aligned}\right\} \tag{7}$$

and

$$\left.\begin{aligned} &1.)\quad w_1\Big|_{r=0} = w_2\Big|_{r=0} = 0 \\ &2.)\quad w_1'\Big|_{r_0} = \frac{D_1 - h_1 r_0}{D_1 r_0}\, w_1\Big|_{r_0} \\ &\qquad w_2'\Big|_{r_0} = \frac{D_2 - h_2 r_0}{D_2 r_0}\, w_2\Big|_{r_0} \end{aligned}\right\}. \tag{8}$$

The system (6) is linear and homogeneous, and its boundary conditions (7) are non-homogeneous. Its solution, which is immediate, is

$$\left.\begin{aligned} y_1 &= F \sin \mu_1 r + G \sin \mu_2 r \\ y_2 &= \xi(\mu_1) F \sin \mu_1 r + \xi(\mu_2) G \sin \mu_2 r \end{aligned}\right\} \tag{9}$$

where $\mu_1$ and $\mu_2$ are roots (assumed distinct) of

$$\begin{vmatrix} A_{11} - D_1\mu^2 & A_{12} \\ A_{21} & A_{22} - D_2\mu^2 \end{vmatrix} = 0, \tag{10}$$

$$\xi(\mu) = \frac{D_1\mu^2 - A_{11}}{A_{12}} = \frac{A_{21}}{D_2\mu^2 - A_{22}},$$

and

$$\left.\begin{aligned} F &= \frac{\xi(\mu_2)\alpha_{22}h_1r_0D_2c_{01} - \alpha_{12}h_2r_0D_1c_{02}}{D_1D_2\{\xi(\mu_2)\alpha_{11}\alpha_{22} - \xi(\mu_1)\alpha_{12}\alpha_{21}\}} \\ G &= \frac{\alpha_{11}h_2r_0D_1c_{02} - \xi(\mu_1)\alpha_{21}h_1r_0D_2c_{01}}{D_1D_2\{\xi(\mu_2)\alpha_{11}\alpha_{22} - \xi(\mu_1)\alpha_{12}\alpha_{21}\}}, \end{aligned}\right\} \tag{11}$$

where

$$\alpha_{ij} = \mu_j \cos \mu_j r_0 - \frac{D_i - h_i r_0}{D_i r_0} \sin \mu_j r_0.$$

B. Time dependent equation:

It proves to be convenient in discussing the time dependent equation to change the units of $t$ so that $D_1 = p$, $D_2 = 1/p$, $p > 1$ if $D_1 > D_2$. The new parameter $p$ will satisfy these conditions if we put

$$t_{\text{new}} = \frac{D_1}{p} t_{\text{old}}, \qquad p^2 = \frac{D_1}{D_2}.$$

In these units our time dependent equations are

$$\left.\begin{aligned} \frac{1}{p}\dot{w}_1 - w_1'' &= a_{11}w_1 + a_{12}w_2 \\ p\dot{w}_2 - w_2'' &= a_{21}w_1 + a_{22}w_2 \end{aligned}\right\} \tag{12}$$

where $a_{11} = A_{11}/D_1$, $a_{21} = A_{21}/D_2$, etc.

Our problem now is to determine solutions of (12) which satisfy the homogeneous boundary conditions (8). First we can show that no undamped periodic solutions of (12) exist if $a_{12}$ and $a_{21}$ have the same sign. To this end we assume a solution of (12) of the form

$$\left.\begin{aligned} w_1 &= \sqrt{\pm\, a_{12}}[\psi_1(r) - \psi_2(r)]e^{\lambda t} \\ w_2 &= \sqrt{\pm\, a_{21}}[\psi_1(r) + \psi_2(r)]e^{\lambda t} \end{aligned}\right\} \tag{13}$$

(where $-$ sign is used if $a_{12}$, $a_{21} < 0$), and substitute into (12). The resulting equations and boundary conditions are

$$\left.\begin{aligned} \psi_1'' &= M_{11}\psi_1 + M_{12}\psi_2 + \lambda N_{11}\psi_1 + \lambda N_{12}\psi_2 \\ \psi_2'' &= M_{21}\psi_1 + M_{22}\psi_2 + \lambda N_{21}\psi_1 + \lambda N_{22}\psi_2 \end{aligned}\right\} \tag{14}$$

and

$$\left.\begin{aligned} &1.) && \psi_1\Big|_{r=0} = \psi_2\Big|_{r=0} = 0 \\ &2.) && \psi_1'\Big|_{r_0} + k_1\psi_1\Big|_{r_0} + k_2\psi_2\Big|_{r_0} = 0 \\ & && \psi_2'\Big|_{r_0} + k_2\psi_1\Big|_{r_0} + k_1\psi_2\Big|_{r_0} = 0 \end{aligned}\right\}. \tag{15}$$

The constants $M_{ij}$, $N_{ij}$, and $k_1$ are real functions of the $a$'s, $h$'s and $p$; for our purposes it is sufficient to note that

$$M_{12} = M_{21},$$
$$N_{11} = N_{22} = 1/2(p + 1/p),$$
$$N_{12} = N_{21} = 1/2(p - 1/p).$$

These relations insure that system (14) is "self-adjoint" (Hilbert, 1921)—hence all the "characteristic numbers" $\lambda$ are real. Since the $\lambda$'s occur in the exponential in (13), if they are all real no periodicities are possible.

The transformation (13) is not real in case $a_{12}$ and $a_{21}$ have opposite signs and so this method cannot then be used to prove anything about the $\lambda$'s. However, if we make the further simplifying assumption that $h_1/D_1 = h_2/D_2$ we can solve (12) completely.*

First we make the substitution in (12) and (8):

$$\left.\begin{aligned} w_1 &= \theta e^{lt} z_1(r, t) \\ w_2 &= e^{lt} z_2(r, t) \end{aligned}\right\} \tag{16}$$

where

$$\theta^2 = -\frac{a_{12}}{a_{21}}, \qquad \frac{a_{12}}{\theta} = -\theta a_{21}, = b,$$

and $l$ and $a$ are chosen so that

$$\left.\begin{aligned} 2l &= pa_{11} + \frac{1}{p} a_{22} \\ 2a &= pa_{11} - \frac{1}{p} a_{22} \end{aligned}\right\}. \tag{17}$$

The resulting equation is

$$\left.\begin{aligned} \frac{1}{p}\dot{z}_1 - z_1'' &= \frac{1}{p} a z_1 + b z_2 \\ p\dot{z}_2 - z_2'' &= -b z_1 - p a z_2 \end{aligned}\right\} \tag{18}$$

and the boundary conditions (8) are still satisfied by $z_i$. We assume that $z_i$ is the product of an exponential time and an exponential space factor so that $\dot{z}_i = \lambda z_i$, $z_i'' = \kappa^2 z_i$. Putting these expressions into (18) we find

$$\left.\begin{aligned} [\kappa^2 - (\lambda - a)/p] z_1 + b z_2 &= 0 \\ -b z_1 + [\kappa^2 - p(\lambda + a)] z_2 &= 0 \end{aligned}\right\}. \tag{19}$$

For a non-trivial solution we must have $z_2 = \xi z_1 \neq 0$; thus

$$\left.\begin{aligned} \kappa^2 - (\lambda - a)/p &= -b\xi \\ \kappa^2 - p(\lambda + a) &= b/\xi \end{aligned}\right\}. \tag{20}$$

* That in many cases such an assumption is reasonable is shown by J. Reiner (1938).

This pair of equations, when solved for $\lambda$ and $\kappa^2$, yield

$$\begin{aligned}\lambda &= -\left[A(p + 1/p) + B(\xi + 1/\xi)\right],\\ \kappa^2 &= -\left[2A + B(p\xi + 1/p\xi)\right],\end{aligned} \tag{21}$$

where

$$A = \frac{a}{p - 1/p}, \qquad B = \frac{b}{p - 1/p}.$$

The value of $\lambda$ is unchanged if we substitute $\xi' = 1/\xi$; on the other hand, if $\xi \neq \pm 1$, we get two different values for $\kappa$, $\kappa_1$ and $\kappa_2$, corresponding to $\xi$ and $1/\xi$. This means that two linearly independent solutions of (18) corresponding to the same $\lambda$ are

$$\left.\begin{aligned} z_{11} &= e^{\lambda t}e^{\kappa_1 r}\\ z_{21} &= \xi e^{\lambda t}e^{\kappa_1 r}\end{aligned}\right\} \tag{22}$$

and

$$\left.\begin{aligned} z_{12} &= e^{\lambda t}e^{\kappa_2 r}\\ z_{22} &= \frac{1}{\xi}\, e^{\lambda t}e^{\kappa_2 r}\end{aligned}\right\} \tag{23}$$

where

$$\left.\begin{aligned} \kappa_1^2 &= -\left[2A + B(p\xi + 1/p\xi)\right]\\ \kappa_2^2 &= -\left[2A + B(p/\xi + \xi/p)\right]\end{aligned}\right\}. \tag{24}$$

The general solution of (18) may now be written

$$\left.\begin{aligned} z_1 &= (\alpha e^{\kappa_1 r} + \beta e^{-\kappa_1 r} + \gamma e^{\kappa_2 r} + \delta e^{-\kappa_2 r})e^{\lambda t}\\ z_2 &= \left(\xi\alpha e^{\kappa_1 r} + \xi\beta e^{-\kappa_1 r} + \frac{\gamma}{\xi}\, e^{\kappa_2 r} + \frac{\delta}{\xi}\, e^{-\kappa_2 r}\right)e^{\lambda t}\end{aligned}\right\}. \tag{25}$$

The $\xi$ and $\lambda$ are still undetermined; they are to be found by applying the boundary conditions (8) which we shall rewrite for convenience:

$$\left.\begin{aligned} &1.)\quad z_1\Big|_{r=0} = z_2\Big|_{r=0} = 0\\ &2.)\quad z_1'\Big|_{r_0} + s_1 z_1\Big|_{r_0} = 0\\ &\qquad\; z_2'\Big|_{r_0} + s_2 z_2\Big|_{r_0} = 0\end{aligned}\right\}. \tag{26}$$

In these equations $s_i$ is an abbreviation for $(h_i r_0 - D_i)/D_i r_0$; $s_1 = s_2$ by the assumption $h_1/D_1 = h_2/D_2$. Putting (25) into (26) we obtain the following system:

$$\left.\begin{aligned}
\alpha + \quad & \beta + \quad & \gamma + \quad & \delta = 0 \\
\xi\alpha + \quad & \xi\beta + \quad & \frac{1}{\xi}\gamma + \quad & \frac{1}{\xi}\delta = 0 \\
(\kappa_1 + s_1)e^{\kappa_1 r_0}\alpha + (s_1 - \kappa_1)e^{-\kappa_1 r_0}\beta + (\kappa_2 + s_1)e^{\kappa_2 r_0}\gamma + (s_1 - \kappa_2)e^{-\kappa_2 r_0}\delta &= 0 \\
\xi(\kappa_1 + s_1)e^{\kappa_1 r_0}\alpha + \xi(s_1 - \kappa_1)e^{-\kappa_1 r_0}\beta + (\kappa_2 + s_1)e^{\kappa_2 r_0}\gamma/\xi + (s_1 - \kappa_2)e^{-\kappa_2 r_0}\delta/\xi &= 0
\end{aligned}\right\} . \quad (27)$$

These equations are homogeneous in $\alpha$, $\beta$, $\gamma$, and $\delta$. They will have a non-trivial solution if and only if the determinant of coefficients vanishes. This condition may be written

$$(\xi - 1/\xi)^4(\kappa_1 \cosh \kappa_1 r_0 + s_1 \sinh \kappa_1 r_0)(\kappa_2 \cosh \kappa_2 r_0 + s_1 \sinh \kappa_2 r_0) = 0, \quad (28)$$

as can be shown by simple algebra. The values of $\lambda$ ($\kappa_i$ are functions of $\lambda$) which satisfy this equation are the required characteristic values. Excluding the case $\xi = \pm 1$ (since, as mentioned before, this case does not give enough linearly independent solutions), we find

$$\frac{\kappa_1}{s_1} = - \tanh \kappa_1 r_0, \quad (29)$$

or

$$\frac{\kappa_2}{s_1} = - \tanh \kappa_2 r_0. \quad (30)$$

Equations (29) and (30) are of standard form (Byerly, 1894) and can be solved graphically for $\kappa_1$ and $\kappa_2$. It is convenient to this end to put $\kappa = im$ where $m$ is real; then

$$m = s_1 \tan m r_0 \quad (30A)$$

in either case. The characteristic values $\lambda$ fall into two classes: those for which $\kappa_1 = im$ and those for which $\kappa_2 = im$. There is no a priori reason to expect these two classes of $\lambda$ to be the same. To determine the $\lambda$'s of class 1, we substitute $\kappa_1 = im$ in (24):

$$m^2 = 2A + B(p\xi + 1/p\xi)$$

or

$$p\xi = \frac{m^2 - 2A}{2B} \pm \sqrt{(m^2 - 2A)^2/4B^2 - 1}$$

and

$$\frac{1}{p\xi} = \frac{m^2 - 2A}{2B} \mp \sqrt{(m^2 - 2A)^2/4B^2 - 1}.$$

Thus

$$B(\xi + 1/\xi) = (p + 1/p)(m^2 - 2A)/2 \pm (p - 1/p)\sqrt{(m^2/2 - A)^2 - B^2}$$

and substituting this expression into (21) we find

$$\lambda = -m^2(p + 1/p)/2 \mp (p - 1/p)\sqrt{(m^2/2 - A)^2 - B^2}. \tag{31}$$

In the same way by substituting $\kappa_2 = im$ in (24) we find the $\lambda$'s of class 2 to be given also by expression (31), so that the two classes of characteristic $\lambda$'s fall together. It may be remarked that if $s_1 \neq s_2$ so that equation (28) does not break into two factors, this degeneracy is removed.

Since there are infinitely many characteristic functions (corresponding to the infinity of $\lambda$'s) we can expect that the complete solution of (2) will be an infinite series of the same general sort that enters in the solution of the vibrating string problem. The validity of the so-called expansion theorem, by virtue of which any initial concentration can be expressed as an infinite sum of characteristic functions, can be demonstrated, but the proof is somewhat lengthy, and so will be omitted. The complete solution of (2) thus turns out to be

$$\left.\begin{aligned} c_1(r, t) &= \frac{1}{r}\left\{\sum_{i=1}^{\infty} E_{1i} \sin m_i r e^{(\lambda_i + l)t} + F \sin \mu_1 r + G \sin \mu_2 r\right\} \\ c_2(r, t) &= \frac{1}{r}\left\{\sum_{i=1}^{\infty} E_{2i} \sin m_i r e^{(\lambda_i + l)t} + \xi(\mu_1) F \sin \mu_1 r + \xi(\mu_2) G \sin \mu_2 r\right\} \end{aligned}\right\}, \tag{32}$$

where

$$E_{ji} = \left\{\left[2(m_i r_0)^2 (D/h)^2 + (r_0 - D/h)^2\right] / \left[r_0 (m_i r_0)(D/h)^2 + r_0^2 - (D/h)^2\right]\right\} \times \int_0^{r_0} \left[r c_j(r, 0) - y_j(r)\right] \sin m_i r dr,$$

and expressions for all other constants have already been given.

C. Transition to the limit:

Rashevsky has shown that in the limiting case of infinite diffusion coefficients the concentrations are exponential functions of the time of the form $e^{\Lambda t}$, where

$$\Lambda = -\frac{1}{2}\left\{\left(\frac{3h_1}{r_0} - A_{11} + \frac{3h_2}{r_0} - A_{22}\right) \pm \sqrt{\left(\frac{3h_1}{r_0} - A_{11} + \frac{3h_2}{r_0} - A_{22}\right)^2 - 4\left[\left(\frac{3h_1}{r_0} - A_{11}\right)\left(\frac{3h_2}{r_0} - A_{22}\right) - A_{12}A_{21}\right]}\right\}.$$

As a check on the correctness of our results we shall show that for large $D$ the concentrations given in (32) obey an exponential law with this same expression for $\Lambda$.

By (30A)

$$\frac{D_1}{D_1 - r_0 h_1} m_i r_0 = \tan m_i r_0; \tag{33}$$

hence as $D_1$ (and $D_2 = h_2 D_1/h_1$) approaches $\infty$, $D_1/(D_1 - r_0 h_1)$ approaches 1 and

$m_1r_0 = \tan m_1r_0$. This implies that the first non-zero root, $m_1$, of (30A) becomes small as $D$ becomes large. Expanding $\tan m_1r_0$ in Taylor's series,

$$D_1(m_1r_0) = (D_1 - r_0h) \tan m_1r_0 = (D_1 - r_0h)[m_1r_0 + (m_1r_0)^3/3 + \cdots]$$

or since $D_1 \gg r_0h$, we can neglect terms involving $r_0h$ multiplied by the third or higher power of $m_1r_0$ and so

$$D_1(m_1r_0)^3/3 \cong h_1m_1r_0^2,$$

or

$$m_1^2 \cong 3h_1/D_1r_0.$$

If this expression for $m_1^2$ is put into (31) and the result simplified, it is found that

$$\begin{aligned}\lambda_1 &= -\tfrac{1}{2}\{(3h_1+3h_2)/r_0 \\ &\quad \pm\sqrt{(-A_{11}-A_{22}+[3h_1+3h_2]/r_0)^2-4[(A_{11}-3h_1/r_0)(A_{22}-3h_2/r_0)-A_{12}A_{21}]}\} \\ &= -\tfrac{1}{2}\{(3h_1+3h_2)/r_0 \pm \sqrt{R}\},\end{aligned}$$

where $R$ stands for the expression under the radical. Since $l = \frac{1}{2}(A_{11}+A_{22})$, the first harmonic in (32) has its period determined by

$$\lambda_1 + l = -\tfrac{1}{2}\{-A_{11} - A_{22} + (3h_1 + 3h_2)/r_0 \pm \sqrt{R}\}$$

which is the same as $\Lambda$. The space dependent part of the first harmonic in (32) reduces to a constant, since the coefficients $E_{ji}$ are constants and $(\sin m_1r)/r$ approaches $m_1$ as $m_1$ becomes small.

As far as the higher harmonics in (32) are concerned, we simply note that from (33) $m_i$ $(i>1)$ is finite and so $Dm_i^2$ becomes infinite with $D$. Also, examination of the normalizing factor $1/N_i$ in the expressions for $E_{ji}$ in case the $D$'s are large, shows that

$$\frac{1}{N_i} = 2(h/D)^2[(m_ir_0)^2 + 1]/r_0(h/D)^2[(m_ir_0)^2 - 1]$$

which is always finite. Since $\lambda_i$ contains a term $-m_i^2(h_1D_1+h_2D_2/h_1)$, as $D$ becomes large, this term becomes large, and the exponential $e^{\lambda_i t}$ becomes negligible.

*Discussion of results*

In the old units the exponential factor in the first harmonic can be written $e^{(\lambda_1+l)t}$, where

$$\begin{aligned}\lambda_1+l &= \tfrac{1}{2}(A_{11}+A_{22}) - [m_1^2(D_1+D_2)/2] \\ &\quad \mp (D_1-D_2)\sqrt{[m_1^2/2-(A_{11}-A_{22})/2(D_1-D_2)]^2 - |A_{12}A_{21}|/(D_1-D_2)^2}. \quad (34)\end{aligned}$$

For an undamped, periodic first harmonic, $(\lambda_1+l)$ must be a pure imaginary. This will be so provided the constants $A_{ij}$ and $D_i$ satisfy

$$A_{11} + A_{22} - m_1^2(D_1 + D_2) = 0, \quad (35)$$

and

$$|A_{12}A_{21}|/(D_1 - D_2)^2 > [m_1^2/2 - (A_{11} - A_{22})/2(D_1 - D_2)]^2. \quad (36)$$

Since the $m_i$ increases monotonically, if the first harmonic is undamped all higher harmonics will be damped, and so, for any given set of constants, at most, one undamped harmonic can occur.

The condition (35) which must be satisfied for undamped periodicity is a very stringent one, and we would expect it to hold only in rare cases. Ordinarily the oscillations will be damped, and only relation (36) will be satisfied. This means that $A_{12}A_{21}$ and $(A_{11}-A_{22})^2$ are of the same order at least as $D^2m_1^4$. We can reasonably assume both $A_{12}A_{21}$ and $(A_{11}-A_{22})^2$ to be of approximately the same order as the square of any one reaction constant, say $A_{ij}$. Also, since $0<m_1<\pi/2r_0$, for ordinary values of $h$, $r_0$, and $D$ we would expect $m\sim 1/r_0$. Relation (36) thus implies

$$A_{ij} \geq D/r_0;$$

that is for small cells ($r_0$ small), $A_{ij}$ (the rates of reaction) must be large and this, from (34), means short periods. This result is biologically plausible: periodic processes (such as reproduction) in bacteria (small size) have short periods; similarly, high rates of metabolism are rather the rule in bacteria.

The dependence of period on temperature can be found roughly by assuming, as a first approximation, that the reaction constants satisfy the Arrhenius relation, $A_{ij}=k_{ij}e^{-\mu/RT}$, and that the $D_i$ and $h_i$ are sensibly constant. The $A_{ij}$ increases with $T$ and from the previous remarks this implies a decrease in period with increase in temperature. This result is in accordance with the generally observed increase of biological frequencies with temperature.*

### *Numerical examples*

The order of magnitude of the periods obtained when biologically plausible values of the constants are used can be indicated by the following two examples:

Case I:

If

$$D_1 = 6 \times 10^{-7}\ \text{cm.}^2\ \text{sec.}^{-1} \qquad D_2 = 3 \times 10^{-7}\ \text{cm.}^2\ \text{sec.}^{-1}$$

$$h_1 = 2 \times 10^{-4}\ \text{cm. sec.}^{-1} \qquad h_2 = 10^{-4}\ \text{cm. sec.}^{-1}$$

$$A_{11} = \pi^2/4 \times 10^{-1}\ \text{sec.}^{-1} \qquad A_{12} = -4 \times 10^{-1}\ \text{sec.}^{-1}$$

$$A_{21} = 4 \times 10^{-1}\ \text{sec.}^{-1} \qquad A_{22} = 0$$

$$r_0 = 3 \times 10^{-3}\ \text{cm.}$$

then, from (30A),

$$m_1 = \pi/6 \times 10^3\ \text{cm.}^{-1},$$

* An interesting discussion of temperature characteristics for Alpha brain waves has been given by Hoagland, Cameron and Rubin (1937), in which the increase in frequency with temperature is pointed out. The mechanism suggested in this paper is of a "relaxation oscillation" type, which is different from our postulated mechanism.

and from (34),

$$\lambda_1 + l \doteq \mp (4/10)i,$$

and the period of the first harmonic is

$$P = 2\pi/|\lambda_1 + l| \doteq 15 \text{ seconds}.$$

All higher harmonics are damped; for example the real part of $\lambda_2+l$ is about $-2$ sec$^{-1}$ and so the second harmonic falls to $1/e$th of its original value in $1/2$ second. The third and higher harmonics are damped even more.

Case II:

If

$$D_1 = 10^{-6} \text{ cm.}^2 \text{ sec.}^{-1} \qquad D_2 = \tfrac{1}{2} \times 10^{-6} \text{ cm.}^2 \text{ sec.}^{-1}$$

$$h_1 = 10^{-4} \text{ cm. sec.}^{-1} \qquad h_2 = \tfrac{1}{2} \times 10^{-4} \text{ cm. sec.}^{-1}$$

$$A_{11} = \tfrac{3}{8}\pi^2 \times 10^{-2} \text{ sec.}^{-1} \qquad A_{12} = \pi/2 \times 10^{-2} \text{ sec.}^{-1}$$

$$A_{21} = \pi \times 10^{-2} \text{ sec.}^{-1} \qquad A_{22} = 0$$

$$r_0 = 10^{-2} \text{ cm.}$$

then

$$m_1 = \pi/2 \times 10^2 \text{ cm.}^{-1},$$

and

$$\lambda_1 + l \doteq \pi/2 \times 10^{-2} i,$$

so that the period of the first harmonic is

$$P \doteq 400 \text{ seconds}.$$

In this case the second harmonic falls to $1/e$th of its original value in $1/3$ second.

Comparison of the periods in these two cases shows that the larger cell has the longer period as was remarked before.

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## SUMMARY

The possibility of periodic concentration changes in metabolizing cells is shown to exist in the case of an idealized spherical cell in which two coupled reactions involving two substances are going on. The periods of these oscillations are calculated, using plausible biological values for the cell constants (diffusion coefficient, permeability, etc.), and are found to be of the order of a few minutes.

A possible correlation of these theoretical results with the generally observed increase in frequencies with increased temperature or decreased cell size is suggested.

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